Amersham™ WB system

User Manual

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1 Introduction

The Amersham WB system

The Amersham WB system is an integrated system for SDS-PAGE and Western blotting analysis of proteins based on fluorescence detection. Electrophoresis, scanning, transfer and automated probing steps are all performed by the same system. Reagents for pre-labeling and antibody detection, as well as gel and membrane consumables, are available.

The Amersham WB system includes the instrument, the Amersham WB analyzer, Amersham WB software (software) and accessories and consumables. The Amersham WB analyzer comprises of two units, the Amersham WB elpho & scan unit and the Amersham WB western unit.

The system supports many applications, from quick screening of protein composition or abundance to advanced quantitative analysis for comparisons of protein levels between samples. The proteins can be pre-labeled with the dye reagent Amersham WB Cy™5 and can therefore directly be detected in the gel after electrophoresis. In Western experiments, unlabeled or Cy5 pre-labeled protein samples are transferred to membranes, and target proteins can be detected using Cy3 and/or Cy5 conjugated secondary antibodies. The fluorescence based detection gives high sensitivity and a broad dynamic range, and enables multiplex analysis of the same Western blot membrane.

Multiplex analysis of total protein pre-labeled with Cy5 and target detected with Cy3 labeled secondary antibody enables normalization of target signals using total protein in each lane. Easy to use Amersham WB software for controlling the different steps of the workflow and for automated or manual evaluation of your results is part of the system.

In this chapter

This chapter contains the the following sections:

1.1 About this manual

Purpose of this manual

The *User Manual* provides you with the instructions needed to plan, set up, run and evaluate experiments as well as instructions needed to safely operate and maintain the Amersham WB system.

Typographical conventions

Software items are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File:Open*** refers to the ***Open*** command in the ***File*** menu.

Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

1.2 Important user information

Read this before operating the Amersham WB analyzer

All users must read the entire *Operating Instructions* before installing, operating, or maintaining the instrument. Always keep the *Operating Instructions* at hand when operating the Amersham WB analyzer.

Do not operate the Amersham WB analyzer in any other way than described in the user documentation.
If you do, you may be exposed to hazards that can lead to personal injury, and you may cause damage to the equipment.

Intended use

Amersham WB system is a protein electrophoresis and Western blotting system that includes scanning of gel cards and PVDF cards. Proteins can be pre-labeled with Cy5 and can therefore directly be detected in the gel after electrophoresis. In Western experiments, unlabeled or Cy5 pre-labeled protein samples are transferred to membranes, and target proteins can be detected using Cy3 and/or Cy5 conjugated secondary antibodies. The fluorescence-based detection gives high sensitivity and a broad dynamic range, and enables multiplex analysis of the same Western blot membrane.

Amersham WB system is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Prerequisites

In order to operate the system in the way it is intended, the following pre-requisites must be fulfilled:

- You should have a general understanding of how the PC and Microsoft® Windows® work.
- You should understand the concepts of electrophoresis and Western blotting.
- The instrument and software must be installed according to the instructions in *Amersham WB system Operating Instructions*.

Tip: *To read more about the principles and methods of Western blotting and for guidance on how to perform successful Western blotting, download or order the handbook, Western Blotting – Principles and Methods 28-9998-97, from www.gelifesciences.com.*

Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

Warnings

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

Cautions

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

Notices

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

1.3 Regulatory information and safety instructions

Regulatory information

For regulatory information regarding Amersham WB system, refer to Section 1.3 in *Amersham WB system Operating Instructions*.

Safety instructions

For safety instructions regarding Amersham WB system, refer to Chapter 2 in *Amersham WB system Operating Instructions*.

1.4 Amersham WB system documentation

Introduction

This section describes the user documentation and associated documentation for the Amersham WB system.

User documentation

The table below lists the user documentation delivered with Amersham WB system:

Document	Main contents	Availability
Amersham WB analyzer Unpacking Instruction	This instruction describes how to unpack the Amersham WB analyzer.	Inside the Amersham WB analyzer delivery box.
Amersham WB system Operating Instructions	This manual contains the information needed to: • install, handle and maintain the Amersham WB system in a safe way • perform a typical experiment in a safe way It also contains safety and regulatory information, a description of the system and reference information.	English version in printed format is delivered with the system. Translated versions are available as PDF files on the User Documentation CD.
Amersham WB system User Manual	This manual contains the information needed to: • operate and maintain the Amersham WB system in a safe way • plan, perform and evaluate electrophoresis and Western experiments • troubleshoot the system and results It also contains a description of the system, reference information and ordering information.	Integrated with Amersham WB software as HTML manual in the Help menu. Also available as a PDF file on the User Documentation CD.

Document	Main contents	Availability
Amersham WB software Help	The Amersham WB software Help contains information about: • user workflows for performing electrophoresis and Western experiments • software descriptions • links to the Amersham WB system User Manual for detailed information about handling of the instrument, accessories and consumables	Integrated with Amersham WB software (in the right panel of the software).

Method handbook

The handbook *Western Blotting – Principles and Methods* gives guidance for the complete Western blotting workflow. It describes theoretical and practical aspects of the technique, together with useful hints and tips. The aim of the handbook is to guide and inspire beginners, as well as experts, towards successful Western blotting.

Download or order the handbook, *Western Blotting – Principles and Methods* 28-9998-97, from www.gelifesciences.com.

Data files and application notes

Data files and application notes can be ordered or downloaded from www.gelifesciences.com.

2 System description

About this chapter

Amersham WB system consists of:

- Amersham WB analyzer (two units)
- accessories and consumables to be used with the instrument
- a computer (not included in the delivery)
- Amersham WB software

This chapter describes the different parts of the Amersham WB system.

In this chapter

This chapter contains the following sections:

2.1 Instrument overview

Introduction

This section provides an overview of the Amersham WB analyzer.

The Amersham WB analyzer consists of two units:

- Amersham WB elpho & scan unit
- Amersham WB western unit

The instrument units are controlled from a PC running Amersham WB software.

Note: *The instrument units must always be connected to each other during a run. Both units must be turned on when running an experiment in the Western unit.*

Safety switches

There is high voltage and two lasers inside the Elpho & scan unit. The unit has double safety switches that stop the power to the high voltage converter and lasers when the loader is opened.

There is high voltage inside the transfer tank in the Western unit. The unit has safety switches that stop the power when the transfer tank lid is opened.

Illustration of the Amersham WB analyzer

The illustration below shows the Amersham WB analyzer, with the Elpho & scan unit to the left and the Western unit to the right.

Illustration of the instrument panels

The same setup of indicator lamps are available for each compartment on the instrument units (electrophoresis and scanning, transfer, probing and drying). When the instrument units are turned on, the lamps showing the compartment names are lit. The indicators are located on the instrument panel on each unit.

The illustration below shows the instrument panels on the two units, with the compartment names lit.

Indicators on the instrument

The table below describes the different indicators. The indicators (Ready/run finished, Run and Error or warning) are identical for all instrument unit compartments. In this example, the **TRANSFER** indicators are described.

Status indicators	Status	Description
	Start-up	The text is lit when the unit is starting up and performing internal tests.
	Ready/run finished	The lamp is lit when: • the unit/compartment is ready for a new experiment • a step (i.e., electrophoresis, scanning, transfer, probing or drying) has been completed
	Run	The lamp is lit: • when the unit/compartment is running • during cleaning of the transfer or probing flow paths ***Note:*** *Do not open the related lid(s) while running. For example, opening the transfer lid during transfer will stop the transfer run and may affect the result*.
	Error or warning	The lamp is lit when an error has occurred Refer to the Amersham WB software for information about the error. Suggestions about how to correct the problem are provided by the software.

2.2 Elpho & scan unit

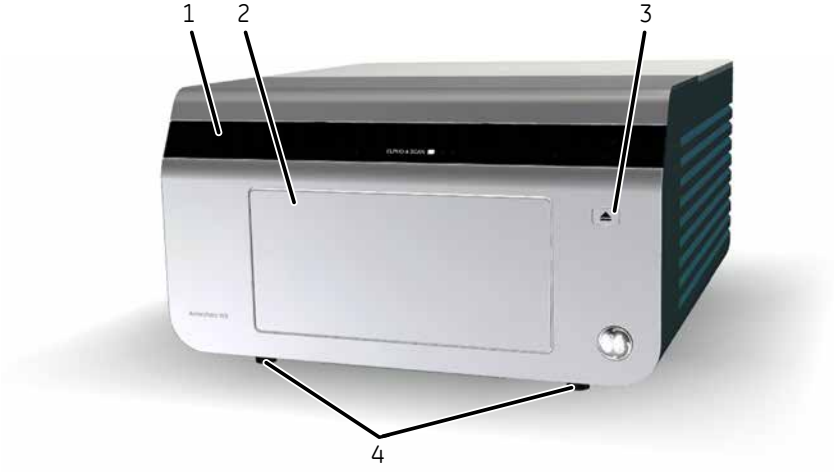
Introduction

The Elpho & scan unit is used to separate SDS treated sample proteins in a polyacrylamide gel. It is also used to scan gels after electrophoresis and PVDF cards after probing and drying in a Western experiment.

This section provides an overview of the Elpho & scan unit.

Illustration of the Elpho & scan unit

The illustration below shows the main parts of the Elpho & scan unit:

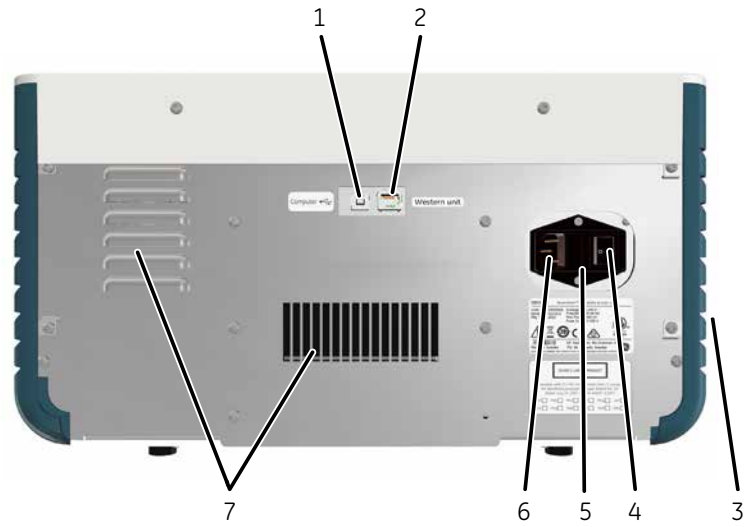


Part	Description
1	Instrument panel (with status indicators)
2	Elpho & scan loader For loading gel cards or PVDF cards.

Part	Description
3	Eject button  To open/close the loader of the electrophoresis and scanning compartment. ***Note:*** *The loader can only be opened when no electrophoresis or scanning experiment is running. Any ongoing run first has to be stopped using the appropriate software instruction to be able to eject the loader.*
4	Adjustable feet (4 feet, one foot at each corner)

Illustration of the Elpho & scan unit rear panel

The illustration below shows the main parts of the Elpho & scan unit rear panel:



Part	Description
1	USB cable connector (USB type B) For connecting the Elpho & scan unit to a computer.
2	Communication connection for the Western unit (use ethernet cable)

Part	Description
3	Air inlet
4	Mains power switch ON=**I**, OFF=**O**
5	Fuse drawer (5x20 mm)
6	Power cord connector (60320/C14)
7	Air outlets

Elpho & scan loader

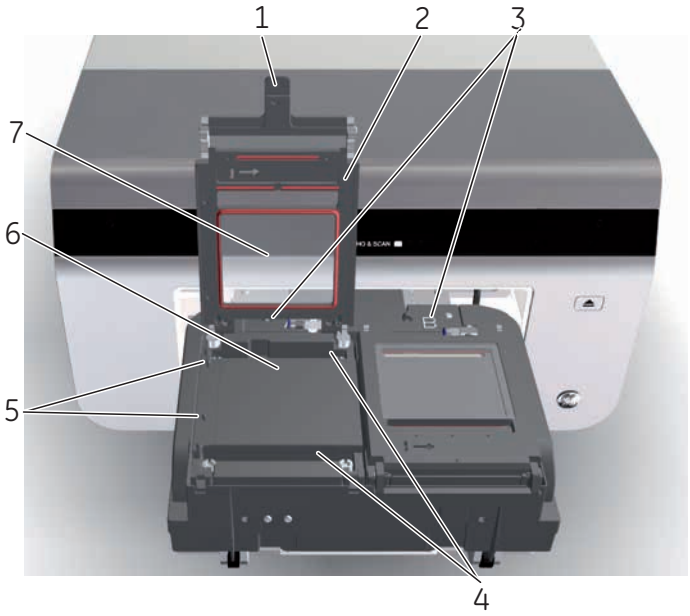
The Elpho & scan loader is used to load:

- gel cards for electrophoresis and scanning
- PVDF cards for scanning. When loading PVDF cards for scanning, an Amersham WB membrane adapter must first be placed on the card plate (see *Section 4.6.9 Scan membrane and view results, on page 224* for more information).

The loader is ejected from the Elpho & scan unit by pressing the eject button on the front panel.

Illustration of the Elpho & scan loader

The illustration below shows the Elpho & scan unit with the loader ejected and the sealing lids of the left card plate opened.



Part	Description
1	Latch For opening the sealing lid.
2	Sealing lid
3	Indicator of position **A** and position **B**
4	Cavities For buffer strip holders with electrical connectors for the buffer electrodes.
5	Guiding pins For correct placement of gel cards or membrane adapters including PVDF cards.
6	Card plate For cooling of gel cards and holding cards flat while scanning.
7	Protective glass

2.3 Western unit

Introduction

The Western unit is used to:

- transfer the separated proteins in the gel to the membrane
- probe the proteins on the membrane with primary antibodies and then secondary antibodies conjugated to a CyDye™
- dry the PVDF cards (before scanning in the Elpho & scan unit)

This section provides an overview of the Western unit.

In this section

This section contains the following subsections:

2.3.1 Overview

Introduction

This section describes an overview of the front and rear panels of the Western unit.

Illustration of the Western unit

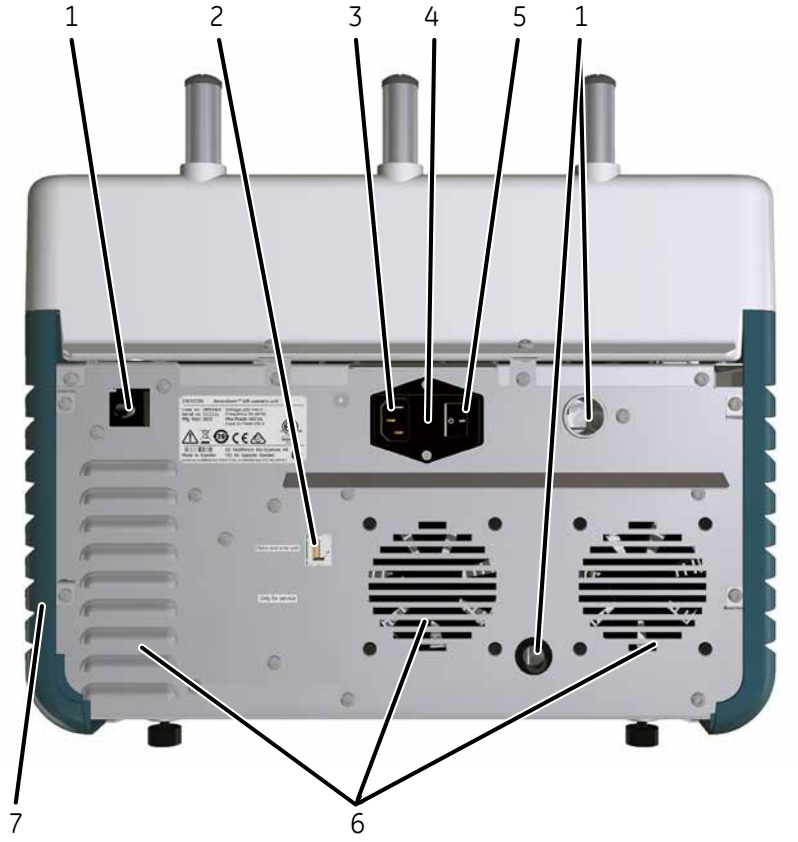
The illustration below shows the main parts of the Western unit:

Part	Description
1	Antibody compartment lid
2	Area pressed to open the antibody compartment lid
3	Instrument panel (for the status indicators)
4	Transfer tank lid

Part	Description
5	Bottle rack (and service compartment lid) ***Note:*** *Only remove the bottle rack when the pumps and tubing are inspected or when parts are replaced as described in Chapter 6 Maintenance, on page 317.*
6	Tubing holders
7	Probing compartment lid
8	Drying compartment lid

Illustration of the Western unit rear panel

The illustration below shows the main parts of the Western unit rear panel:



Part	Description
1	Outlets for waste and overflow tubing
2	Communication connection for the Elpho & scan (use ethernet cable)
3	Power cord connector (60320/C14)
4	Fuse drawer (5x20 mm)
5	Mains power switch (ON=**I**, OFF=**O**)
6	Air outlets
7	Air inlet

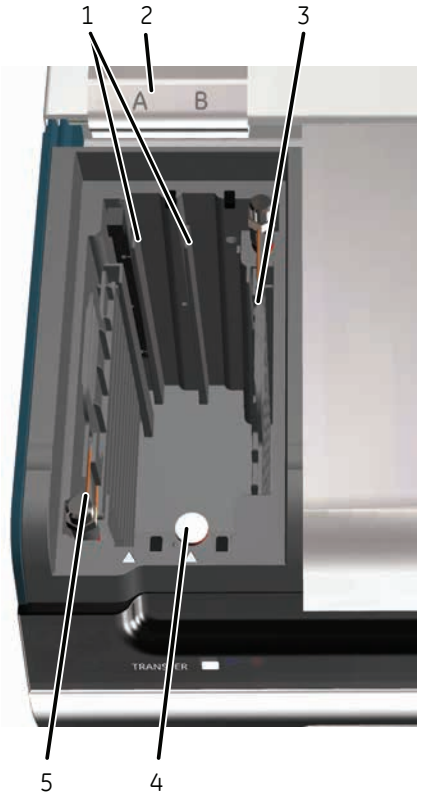
2.3.2 Western unit compartments

Introduction

This section describes the different compartments of the Western unit with the lids opened.

Illustration of the transfer tank

The illustration below shows the transfer tank of the Western unit.

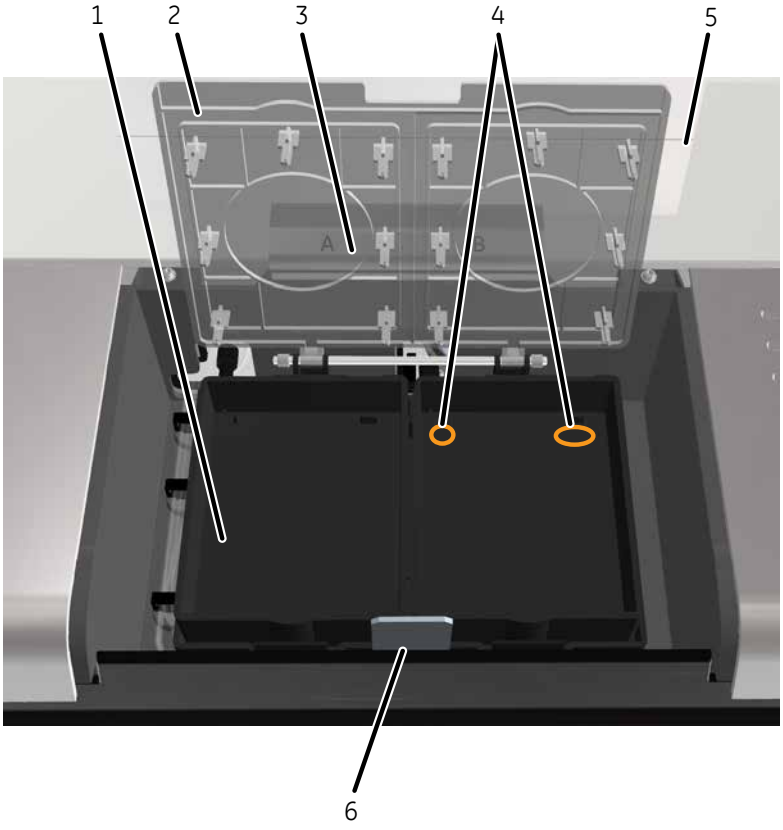


Part	Description
1	Sandwich guiding tracks
2	Indicator of position **A** and position **B**
3	Right transfer electrode

Part	Description
4	Transfer tank filter
5	Left transfer electrode

Illustration of the probing compartment

The illustration below shows the probing compartment of the Western unit.

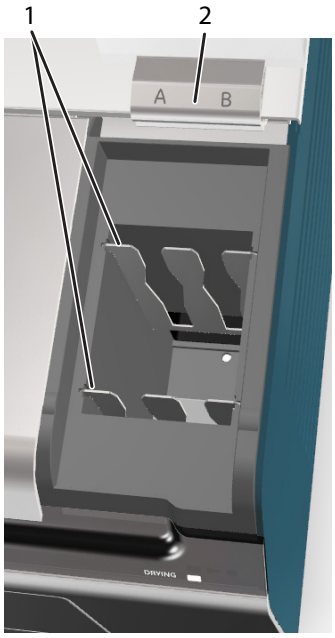


Part	Description
1	Probing chamber
2	Probing chamber lid

Part	Description
3	Indicator of position **A** and position **B**
4	Guiding pins for PVDF card (marked by orange circles)
5	Probing compartment lid
6	Probing chamber lid hatch

Illustration of the drying compartment

The illustration below shows the drying compartment of the Western unit.

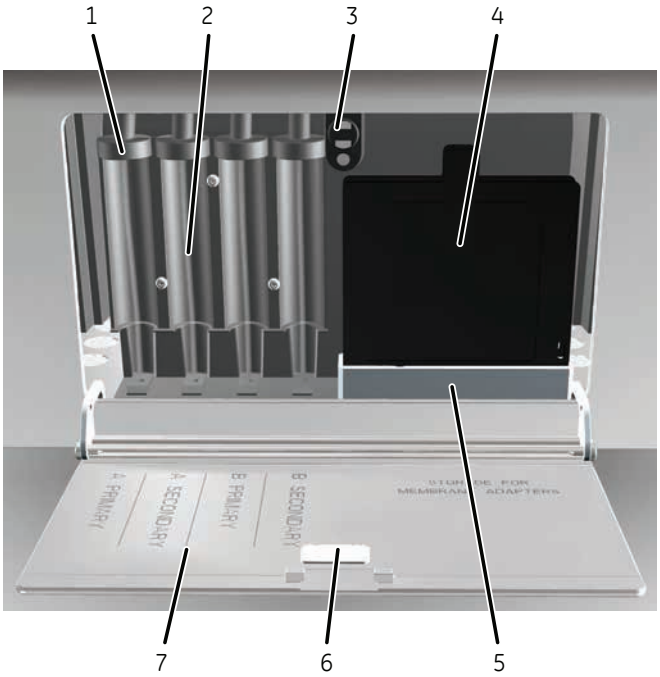


Part	Description
1	Membrane dryer guides
2	Indicator of position **A** and position **B** locations

Illustration of the antibody compartment

The illustration below shows the antibody compartment where 15 ml antibody solution tubes with primary and secondary antibodies are inserted before probing (left side). See *Connect the antibody solution tubes, on page 209* for more information.

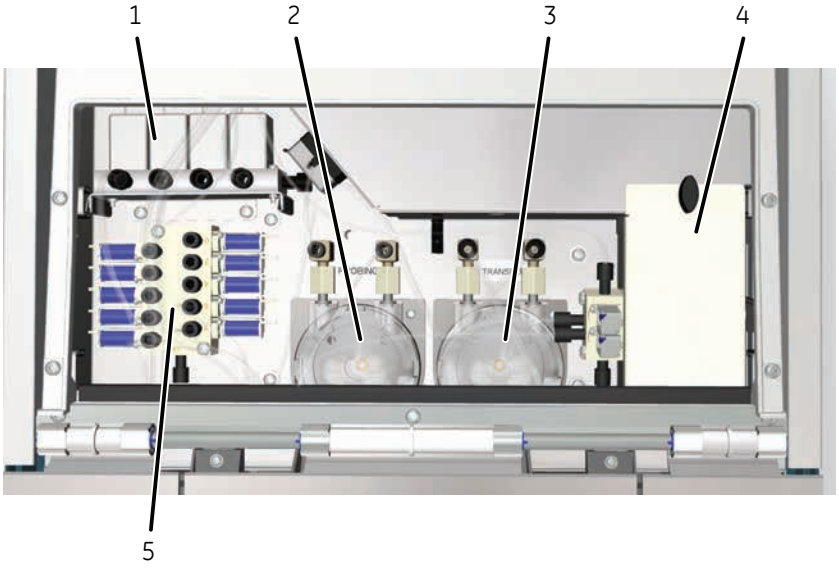
The right side of the compartment is used for storage of the Amersham WB membrane adapters (Membrane adapters).



Part	Description
1	Tube holder
2	Tubing
3	Door lock (magnetic)
4	Membrane adapters
5	Holder for membrane adapters
6	Door lock
7	Indicator of antibody solution tube position **A PRIMARY**, **A SECONDARY**, **B PRIMARY**, and **B SECONDARY**.

Illustration of the service compartment

The illustration below shows the service compartment of the Western unit.



Part	Description
1	Transfer valve block
2	Probing pump
3	Transfer pump
4	Air filter compartment lid
5	Probing valve block

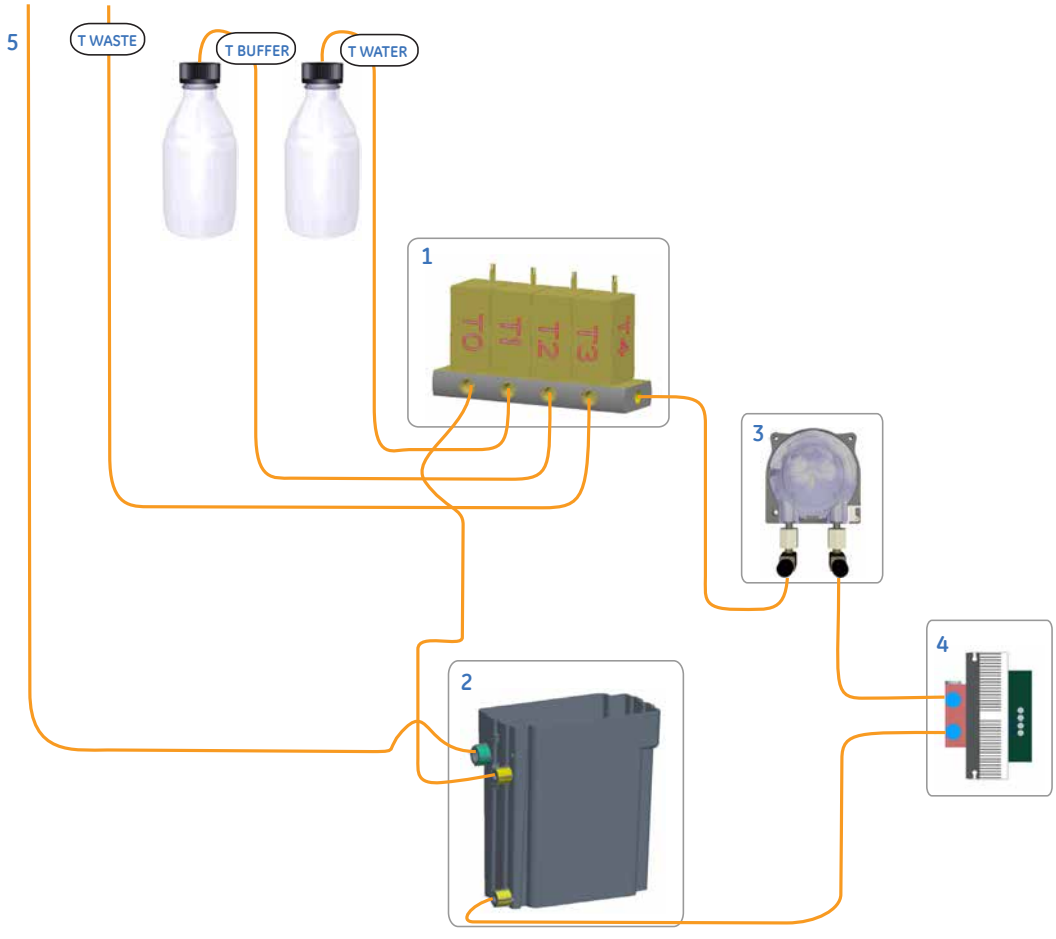
2.3.3 Liquid flow paths

Introduction

This section shows the transfer and probing liquid flow paths in the Western unit and describes the basic principles of operation.

Illustration of the transfer liquid flow path

The illustration below shows a detailed flow chart for the transfer process. The flow chart shows interconnections between instrument components. The white labels refer to tubing labels. The blue numbers refer to instrument components.



The table below describes the components in the flow chart:

Part	Description
1	Transfer valve block For selecting which solution should be pumped through the liquid flow path.
2	Transfer tank
3	Transfer pump For pumping the solution through the liquid flow path.
4	Transfer cooling unit For cooling down the transfer buffer to keep it at room temperature.
5	Transfer tank overflow

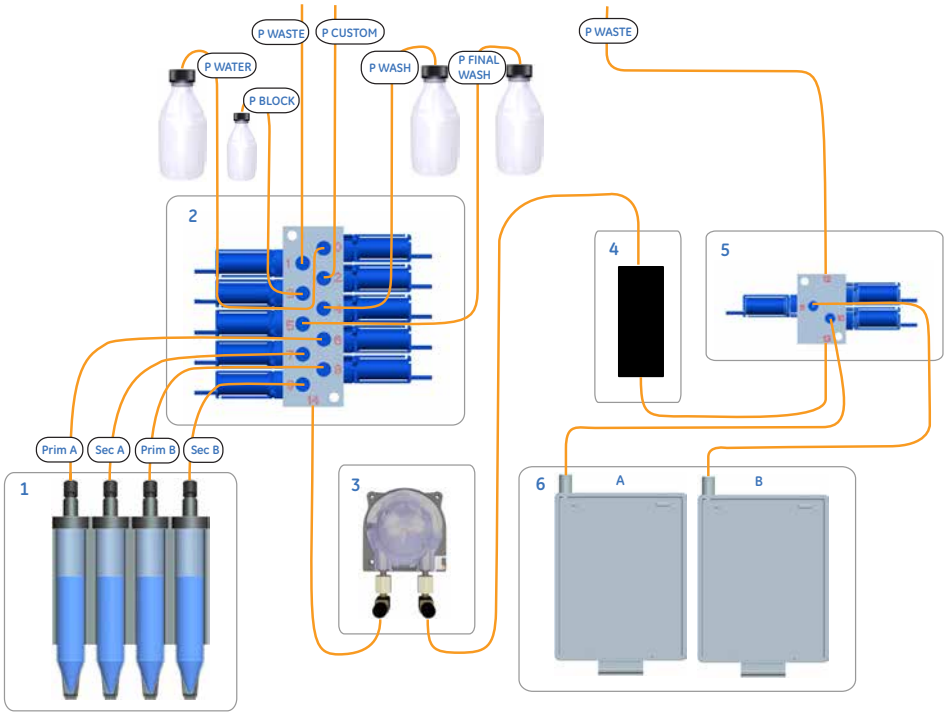
Basic principle of the transfer process

During the transfer, an electrophoretic transfer of the separated proteins in the gel onto the membrane is performed. The basic principle of the transfer process is described below.

Stage	Description
1	When the Amersham WB transfer holder(s) (transfer holder) has been loaded into the transfer tank, the transfer tank is filled with transfer buffer (the tube marked with **T Buffer**).
2	During the transfer step, the transfer buffer is pumped through the transfer tank, transfer cooling unit, transfer pump and the transfer valve block, circulating the transfer buffer during the run. The transfer cooling unit makes sure that the transfer buffer is kept at room temperature during the run.
3	After a run, and after removal of the transfer holders, ultra pure water is used to clean the liquid flow path (the tube marked with **T Water** is used for cleaning).

Illustration of the probing liquid flow path

The illustration below shows a detailed flow chart for the probing process. The flow chart shows interconnections between instrument components. The white labels refer to tubing labels. The blue numbers refer to instrument components.



The table below describes the components in the flow chart:

Part	Description
1	Antibody compartment with antibody solution tubes containing primary and secondary antibodies for positions **A** and **B**.
2	Probing valve block Used to select solution to be pumped through the flow path.
3	Probing pump For pumping the solution through the liquid flow path.
4	Air sensor To detect if air is present in the tubing. The air detector pauses the run if a solution bottle is empty.

Part	Description
5	Probing chamber selector valve To select whether to direct the solution to the probing chambers or from the probing chambers to waste outlet.
6	Probing chambers

Basic principle of the probing process

In probing, primary antibodies are bound to the proteins on the membrane and then secondary antibodies conjugated to a CyDye are bound to the primary antibodies.

The basic principle of the probing process is described below.

Stage	Description
1	The probing chamber is pre-filled with the first solution, as entered in the probing protocol in the software (blocking solution is used as the default). Pre-filling of the probing chamber is started by the user from selecting the appropriate software instruction.
2	The PVDF cards are inserted into the probing chambers and sequential incubations (including wash steps) of membrane with blocking solution, primary antibodies and secondary antibodies are performed.

2.4 Accessories and consumables overview

Introduction

This section gives an overview of the accessories and consumables to be used with the Amersham WB analyzer. It also contains storage information for the consumables and information about how to order consumables.

Accessories and consumables

The table below lists the accessories and consumables to be used with the instrument in relation to the operational task to be performed. The instrument accessories are delivered with the system and the consumables are ordered separately.

Accessories to be installed are described in *Amersham WB system Operating Instructions* and accessories for user maintenance tasks are described in *Maintenance accessories, on page 328*.

For a detailed description of the accessories and consumables, see the subsequent sections.

Task	Required consumables	Required accessories	For details, see...
Pre-labeling of samples	• Amersham WB Cy5 • Amersham WB labeling buffer (Labeling buffer) • Amersham WB loading buffer (Loading buffer) • Amersham WB MiniTrap™ kit (optional)	N/A	Section 2.5
Electrophoresis run	• Amersham WB molecular weight markers (Molecular weight markers) • Amersham WB gel card (Gel card) • Amersham WB buffer strip (Buffer strip) • Amersham WB loading buffer • Amersham WB paper comb (optional) (Paper comb)	Amersham WB buffer strip holder [1] (Buffers strip holder)	Section 2.6

Task	Required consumables	Required accessories	For details, see...
Western blot steps			
Transfer	• Amersham WB gel card from the electrophoresis run • Amersham WB PVDF card (PVDF card) • Amersham WB transfer paper (Transfer paper) • Amersham WB sponge (Sponge) • Laboratory prepared transfer solutions (for recipes, see *Transfer solution recipes, on page 103*)	Amersham WB transfer holder[1] (Transfer holder)	Section 2.7.1
Probing	• Laboratory prepared primary antibody solutions • Secondary antibody: Amersham WB goat anti-mouse Cy3/Cy5 (goat anti-mouse Cy3/Cy5) or Amersham WB goat anti-rabbit Cy3/Cy5 (goat anti-rabbit Cy3/Cy5) • PVDF card from the transfer step • Laboratory prepared probing solutions (for recipes, see *Probing and antibody solution recipes, on page 104*)	Antibody solution tubes[1]	Section 2.7.2
Drying	PVDF card from the probing step	Amersham WB drying holder (Drying holder)	N/A
Scanning of the PVDF card in the Elpho & scan unit	PVDF card from the drying step	Amersham WB membrane adapter[1] (Membrane adapter)	Section 2.7.4
Other			

Task	Required consumables	Required accessories	For details, see...
Scanning data matrix tags on gel cards and PVDF card single packages (optional)	N/A	Matrix tag reader (order separately)	Section 2.8

1 Included in delivery of Amersham WB analyzer.

Order consumables

Depending on the experiments to be performed, different combinations of consumables are required. A selection guide for putting a kit of consumables together is available at www.gelifesciences.com.

For ordering information for separate consumables, see *Chapter 9 Ordering information, on page 360*.

Storage of consumables

The table below summarizes the storage and environmental conditions for the different consumables. The expiry date of the consumables is printed on the package.

Consumable	Storage temperature	Environment
Pre-labeling consumables	-15°C to -30°C	Protect contents from direct sunlight. Avoid repeated freeze-thaw cycles of the Cy5 dye reagent. Diluted Cy5 dye reagent should be used within 30 min and not be frozen.

Consumable	Storage temperature	Environment
Amersham WB molecular weight markers	-15°C to -30°C	Protect the molecular weight (MW) markers from direct sunlight. The MW markers must always be stored in the freezer. Minimize time in room temperature before use and avoid refreezing. MW markers diluted in Loading buffer with DTT can be dispensed in aliquots for single-time use, if frozen directly after dilution. The aliquots should not be stored for longer than one week.
Amersham WB gel card	4°C to 8°C	Store in refrigerator or cold room.
Amersham WB buffer strips	4°C to 8°C	Store in refrigerator or cold room.
Amersham WB PVDF card	Room temperature	Store the PVDF card in the single package, inside the the box in which they are delivered. Store the box in a clean and dry place, protected from light.
Amersham WB transfer paper	Room temperature	Store the transfer papers in a dry place, in the box in which they are delivered. Protect from dust.
Amersham WB paper comb	Room temperature	Store the paper combs in a dry place, in the box in which they are delivered. Protect from dust.
Amersham WB sponge	Room temperature	Protect from dust and direct sunlight.
Amersham WB goat anti-mouse Cy3/Cy5 or Amersham WB anti-rabbit Cy3/Cy5, lyophilized	2°C to 8°C	Protect secondary antibodies from light.

Consumable	Storage temperature	Environment
Reconstituted secondary antibodies (reconstituted in ultra pure water to a concentration of 1 µg/µl)	Store aliquots at -15°C to -30°C	Protect reconstituted secondary antibodies from light. The aliquots should not be stored for longer than 6 months. Avoid repeated freeze-thaw cycles.
Primary antibodies	See manufacturer's instructions.	See manufacturer's instructions.

2.5 Pre-labeling consumables

Introduction

The pre-labeling consumables are used for the pre-labeling of protein samples with a fluorescent dye.

Fluorescent pre-labeling of samples:

- enables total protein detection when scanning the gel card,
- eliminates the need for post-staining the gel.

When to use pre-labeling of proteins

Pre-labeling of proteins is always performed before the electrophoresis step when performing:

- electrophoresis experiments,
- Western experiments with total protein normalization.

Pre-labeling consumables

Pre-labeling consumables are packed in separate foil bags. Each foil bag contains solution reagents sufficient for running 10 gel cards (where 14 lanes/gel card are loaded with pre-labeled samples), that is, 140 individual labeling reactions.

The table below summarizes the contents of the pre-labeling consumables.

Pre-labeling consumable	Content	Amount
Amersham WB Cy5	0.25 mg/ml (approximately 250 pmol/µl) Cy5 dye in DMSO	5 vials (blue caps) 35 µl Cy5 dye solution per vial (enough for two gel cards)
Amersham WB labeling buffer (Labeling buffer)	Tris-based buffer containing SDS with pH 8.7 (at 25°C)	5 vials (black caps) 0.7 ml Labeling buffer solution per vial (enough for two gel cards)
Amersham WB loading buffer (Loading buffer)	50 mM Tris-Cl, 0.25% (w/v) Orange G, 4% SDS, 0.5 mM Lysine	5 vials (orange caps) 0.7 ml Loading buffer solution per vial (enough for two gel cards)

Sample preparation consumable

The table below describes the Amersham WB MiniTrap kit.

Sample preparation consumable	Function	Amount
Amersham WB MiniTrap kit	The kit is designed for rapid and convenient clean-up of protein samples (>5000 M_r) containing interfering substances, e.g., imidazole.	• 30 prepacked disposable PD MiniTrap G-25 columns containing Sephadex G-25 medium • 1 bottle of 10× Amersham WB labeling buffer (14 ml stock solution) • Integrated column stand, waste tray and tube holder

2.6 Accessories and consumables for electrophoresis

Introduction

The following accessories and consumables are needed for protein electrophoresis:

- Amersham WB buffer strip
- Amersham WB buffer strip holder
- Amersham WB molecular weight markers
- Amersham WB gel card
- Amersham WB paper comb, if sample well clean up will be performed
- Amersham WB loading buffer (see *Section 2.5 Pre-labeling consumables, on page 40* for information about the Loading buffer)

In this section

This section contains the following subsections:

2.6.1 Amersham WB molecular weight markers

Introduction

Amersham WB molecular weight markers are a mixture of nine native and recombinant proteins, M_r: 10 000 to 225 000, labeled with both Cy3 and Cy5, for molecular weight determination of proteins on scanned gel cards and PVDF cards.

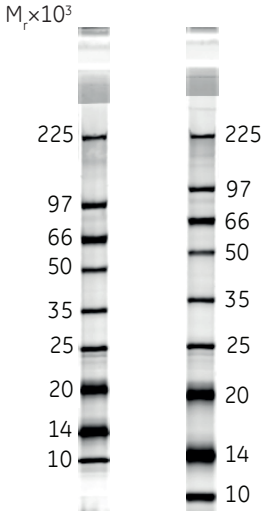
The software calculates molecular weight calibration curves. When two molecular weight calibration curves are used, interpolated data from the curves are used to create sample lane specific calibration curves. The molecular weights of all detected bands in each lane are then calculated.

Content

Each package of Amersham WB molecular weight markers contains solution for running 10 gel cards (where 2 lanes/gel card are loaded with Amersham WB molecular weight markers).

Illustration of MW markers

The illustration below shows the sizes of the Amersham WB molecular weight markers separated on a gradient gel card (left image) and a homogeneous gel card (right image):



MW marker proteins

The table below shows the proteins that are included:

Protein	Molecular weight ($M_r \times 10^3$)
Recombinant protein	225
Phosphorylase b, rabbit muscle	97
Albumin, bovine serum	66
Recombinant protein	50
Recombinant protein	35
Recombinant protein	25
Trypsin inhibitor, soybean	20
Alpha-Lactalbumin, bovine milk	14
Recombinant protein	10

2.6.2 Amersham WB gel card

Introduction

This section describes the gel card details.

Gel card types

There are two types of gel cards:

- Homogeneous gel card: Amersham WB gel card 14, 13.5%
- Gradient gel card: Amersham WB gel card 14, 8-18%

Gel composition

The gels are pre-cast in the gel card casette. The gels are polyacrylamide/bisacrylamide gels containing a Tris-acetate buffer.

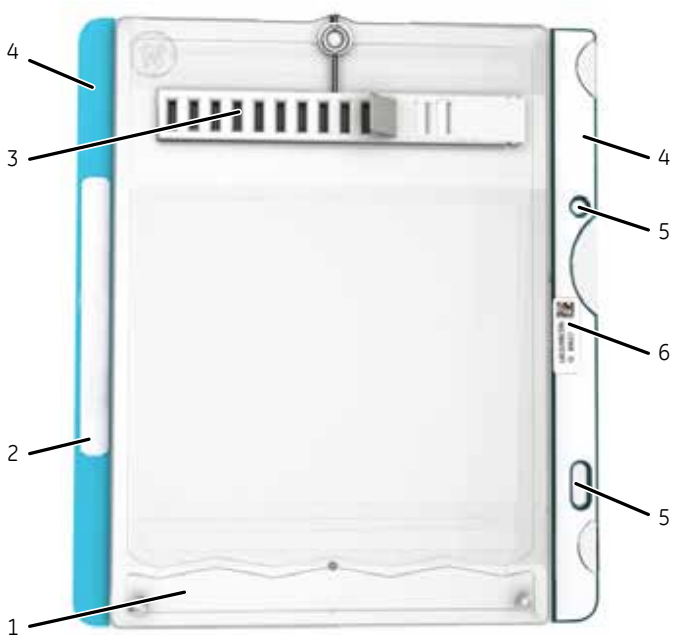
Protein separation resolution

The table below lists the protein separation resolution for the gel card types:

Gel card type	Separation resolution, $M_r \times 10^3$
Amersham WB gel card 14, 13.5%, Homogeneous	10 to 225
Amersham WB gel card 14, 8-18%, Gradient	• 6 to 225 (pre-labeled proteins) • 3.5 to 225 (Western experiments, antibody detection)

Illustration of the front side of a gel card

The illustration below shows the gel card with the front side facing upwards:



Part	Description
1	Writing surface for your own annotation ***Note:*** *Always use a pencil when writing the annotation.* The annotation can be entered in the software as well.
2	Label with gel card type
3	Sample well cover that must be removed when the sealing lid in the Elpho & scan unit has been closed, prior to applying the samples.
4	Gel handles to ease handling of the gel when it has been removed from its support for Western blotting.

Part	Description
5	Guiding holes for the correct placement of the gel cards in the Elpho & scan loader and transfer holder.
6	Label with Data matrix tag, Lot number and ID

Illustration of the reverse side of a gel card

The illustration below shows the gel card with the reverse side facing upwards:

Part	Description
1	Protective films that are removed prior to electrophoresis to enable contact between the gel and buffer strips.
2	Protective film that is removed when preparing the transfer sandwich (only relevant in Western experiments).

2.6.3 Amersham WB buffer strip and Amersham WB buffer strip holder

Introduction

The buffer strip holders hold the buffer strips in place during an electrophoresis run. The buffer strip holders have built-in electrodes, providing contact between the electrode and buffer strip, enabling an electric current for the run.

The buffer strips are loaded into the holders before starting the run and are discarded after the run. The shape of the holder guides the user when placing the buffer strips in the holders, and when placing the holders into the Elpho & scan unit.

See *Preparations before starting electrophoresis, on page 164* for more information.

Buffer used in the buffer strips

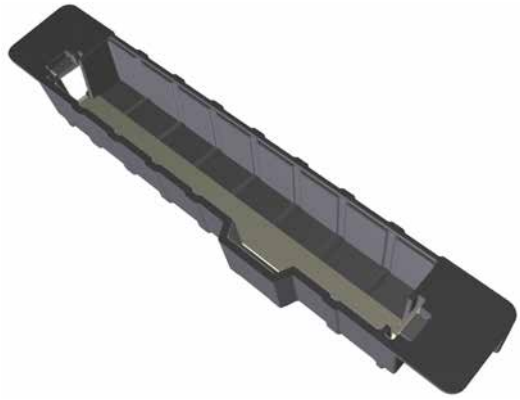
The buffer used in the buffer strips is Tris-tricine.

Illustration of the buffer strips

The illustration below shows a buffer strip.

Illustration of the buffer strip holders

The illustration below shows a buffer strip holder.



2.6.4 Amersham WB paper comb

Introduction

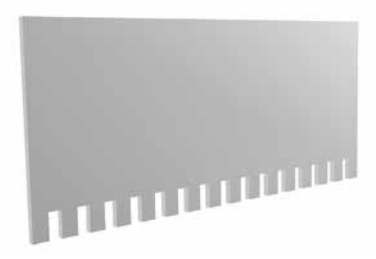
The paper comb is used to clean up the sample wells when proteins have migrated into the gel for a few minutes. Sample well cleanup is recommended to remove excess dye when pre-labeled samples are used. This reduces the background, which improves the detection and quantification of low abundant proteins (<1 ng/band) and low molecular weight proteins (M_r<40 000).

Sample well cleanup will generally improve band resolution, also for unlabeled samples of high protein concentrations or large sample volumes.

For more information about how to use the paper comb, see *Run electrophoresis and gel scanning, on page 169*.

Illustration of the paper comb

The illustration below shows a paper comb.



2.7 Accessories and consumables for Western blot steps

Introduction

This section describes the accessories and consumables used for the transfer of proteins from the gel onto the membrane, the probing of the membrane with primary and secondary antibodies, and the scanning of PVDF cards.

In this section

This section contains the following subsections:

2.7.1 Accessories and consumables for transfer

Introduction

This section describes:

- Amersham WB transfer holder (accessory)
- Amersham WB PVDF card (consumable)
- Amersham WB transfer paper (consumable)
- Amersham WB sponge (consumable)

Amersham WB transfer holder

The transfer holder is used during transfer. It contains the transfer sandwich (sponge, transfer paper, gel card, PVDF card, transfer paper and sponge) prepared before starting transfer. The separated proteins are transferred from the gel to the membrane during the transfer run.

Guiding pins in the transfer holder will make sure that the gel card and PVDF card are positioned correctly when preparing the transfer sandwich.

By using the built-in roller (when moving the roller over the sandwich in the roller tracks), air bubbles will be removed and a reproducible pressure will be applied on the gel card and PVDF card for an even transfer process.

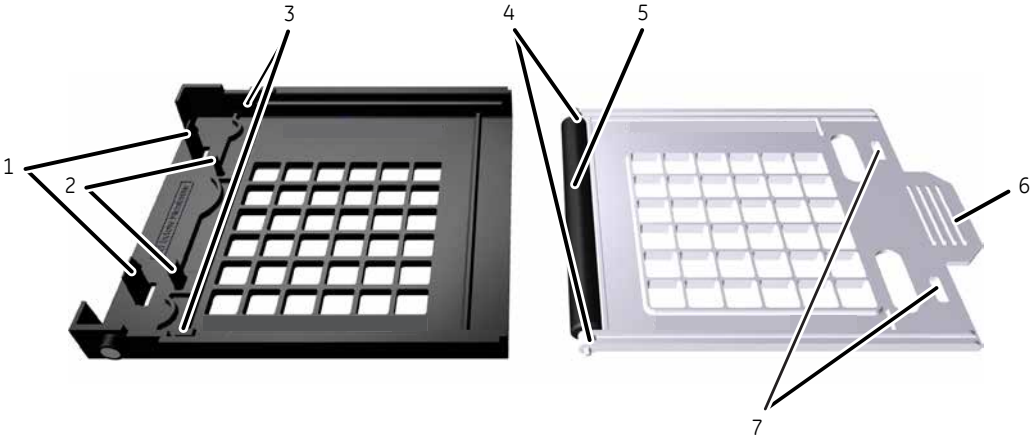
See *Start building the transfer sandwich, on page 192* for more information about how to prepare the transfer sandwich.

Illustration of the transfer holder when closed

The image below shows the transfer holder when it is closed:

Illustration of the transfer holder when disassembled

The image below shows the transfer holder when it has been disassembled.



The table below describes the different parts of the transfer holder.

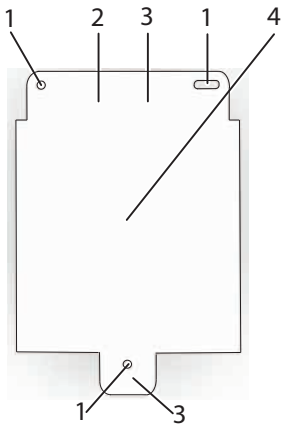
Part	Function
Bottom part (black lid)	
1	Latches

Part	Function
2	Guiding pins for gel card and PVDF card
3	Roller tracks (for roller pins)
Top part (white lid)	
4	Roller pins (fit in roller tracks to assemble the lids)
5	Roller
6	Handle
7	Holes for the latches

Amersham WB PVDF card

In the transfer step, proteins are transferred from the gel to the blotting area of the PVDF card. The PVDF card is a low fluorescent PVDF membrane.

The illustration below shows the different parts of the PVDF card and the PDVF card single package.



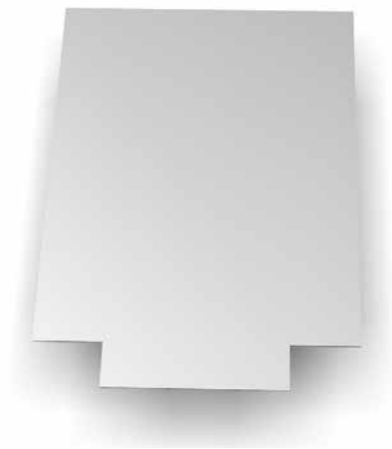
The following table describes the different parts of the PVDF card and PDVF card single package.

Part	Description
1	Guiding holes ***Note:*** *The guiding holes assist the correct placement of the card in the transfer holder, the probing chamber, the drying holder and the Elpho & scan loader.*
2	Writing surface for your own annotation ***Note:*** *Always use a pencil when writing the annotation.* The annotation can be entered in the software as well.
3	Card handles
4	Blotting area
5	PDVF card single package
6	PDVF card with protective papers
7	Label with Product code, Lot number, Identity number and Data matrix tag (contains Product code, Lot number and ID number)

Amersham WB transfer paper

The transfer papers are used when preparing the transfer sandwich.

The illustration below shows a transfer paper:



Amersham WB sponge

Two sponges are used when preparing the transfer sandwich. The sponges need to be replaced after each run.

The illustration below shows a sponge.

2.7.2 Secondary antibodies – probing

Introduction

The target proteins are identified by probing the membrane with primary antibodies and species-specific secondary CyDye conjugated antibodies. By using two different species of primary antibodies (mouse and rabbit) and one Cy3 conjugated secondary antibody and one Cy5 conjugated secondary antibody, a multiplex experiment detecting two proteins simultaneously on the same membrane can be performed.

The following variants of CyDye conjugated secondary antibodies are available:

- Amersham WB goat anti-mouse Cy3 (goat anti-mouse Cy3)
- Amersham WB goat anti-rabbit Cy3 (goat anti-rabbit Cy3)
- Amersham WB goat anti-mouse Cy5 (goat anti-mouse Cy5)
- Amersham WB goat anti-rabbit Cy5 (goat anti-rabbit Cy5)

CyDye (conjugated to the secondary antibodies)

The CyDye conjugated to the secondary antibodies have their own specific excitation and emission wavelengths in the visible light spectra and are spectrally differentiated from each other, resulting in minimum cross-talk, see the table below.

CyDye	Excitation wavelength	Emission wavelength
Cy3	550 nm	570 nm
Cy5	649 nm	670 nm

After excitation, the resulting fluorescent emission signals are captured using the multi-channel fluorescent scanner in the Elpho & scan unit.

The CyDye have a high photostability. The signal on probed membrane is stable for >3 months, if stored in the dark.

Preparation and storage of reconstituted secondary antibodies

Reconstitute the secondary antibodies in 150 µl ultra pure water per vial, to a concentration of 1 µg/µl. Vortex and spin down the reconstituted secondary antibodies.

It is recommended to store the secondary antibodies in aliquots at -15°C to -30°C, and protect from light. Avoid repeated freeze-thaw cycles.

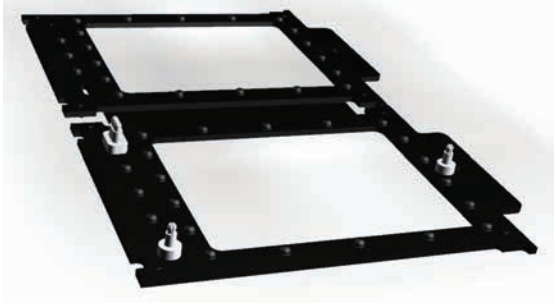
2.7.3 Drying holder – PVDF card drying

Introduction

The Amersham WB drying holder (drying holder) orients and fixes the PVDF card in the correct position for the best possible drying result.

Illustration of the drying holder

The illustrations below show the drying holder when closed (top) and the drying holder when opened (below).



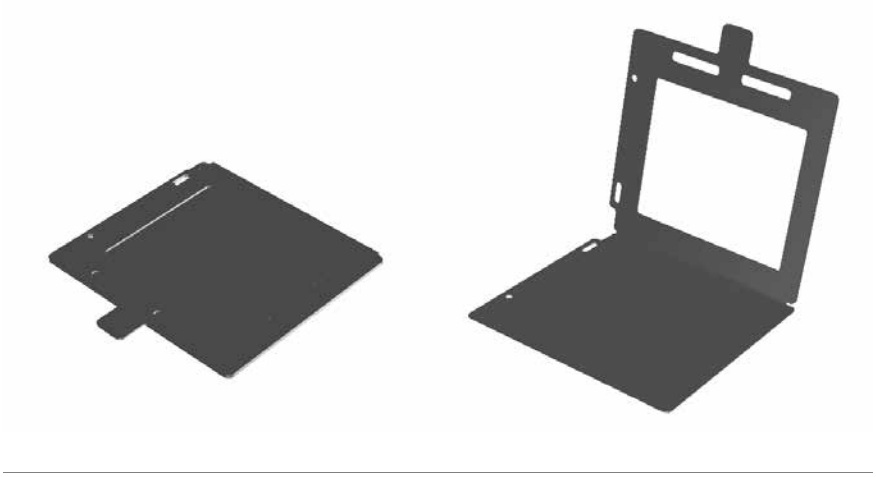
2.7.4 Membrane adapter – PVDF card scan

Introduction

The Amersham WB membrane adapter (membrane adapter) orients and fixes the PVDF card in the correct position for the best possible scanning and detection in the Elpho & scan unit.

Illustration of the membrane adapter

The illustrations below show the membrane adapter when closed (left) and the membrane adapter when opened (right):



2.8 Other accessories

Matrix tag reader

The matrix tag reader is used to enter gel card and PVDF card information (product code, ID and lot number) into the software by scanning the data matrix tags on the gel card and on the PVDF card single package label. It must be ordered separately (see *Chapter 9 Ordering information, on page 360*).

The illustration below shows the matrix tag reader.

Inlet filter holder

The inlet filter holders, with inlet filters, are attached on the inlet tubes that are inserted in the bottles on the bottle rack. Particles are filtered by the inlet filter and the inlet filter holder itself is a weight that holds the tubing in place at the bottom of the bottles.

The illustration below shows an inlet filter holder, with an inlet filter mounted and attached to tubing.

Tubing holders

To compensate for tensions when tubing is placed in bottles, and to avoid bottles falling when liquid level decreases, a tubing holder must be attached to the tubing. See illustration below:

Additional accessories to be installed by the user

The transfer electrodes and the membrane dryer guides must be installed by the user.

See *Amersham WB system Operating instructions* for more information.

Accessories for user maintenance

For a description of accessories for user maintenance, see *Maintenance accessories, on page 328*.

2.9 Amersham WB software overview

About this chapter

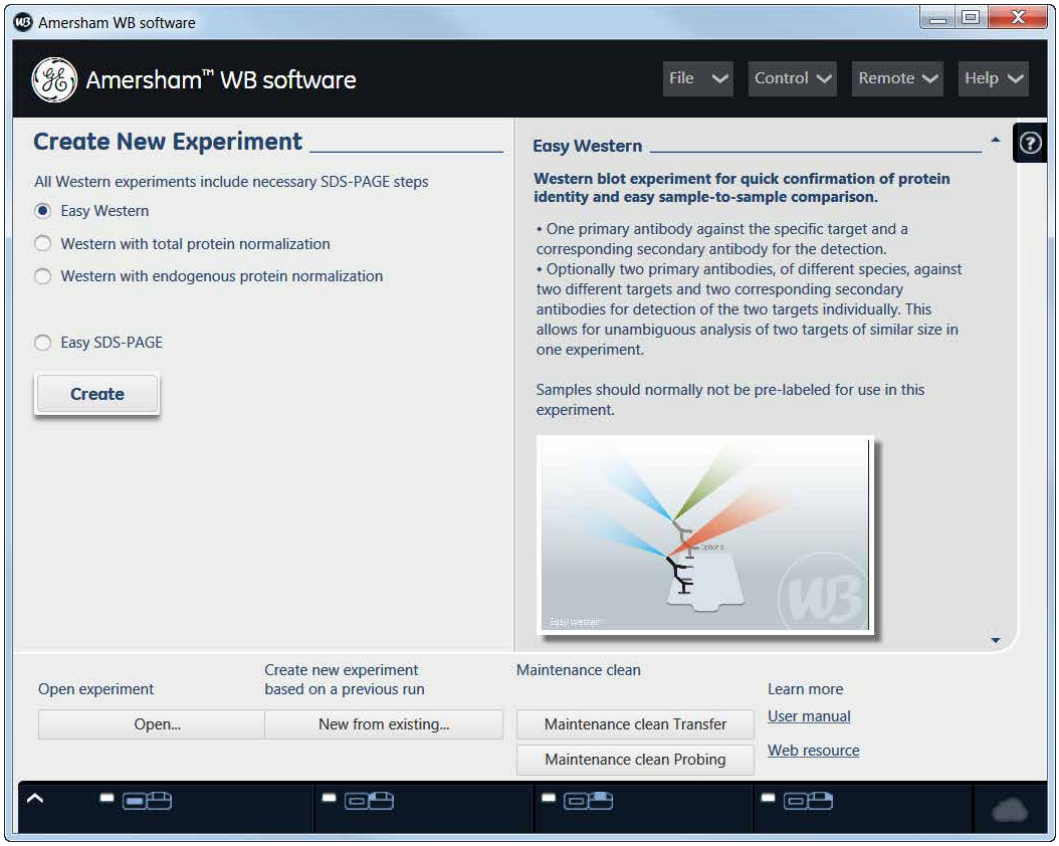
This section gives an overview of the Amersham WB software.

In this chapter

This chapter contains the following sections:

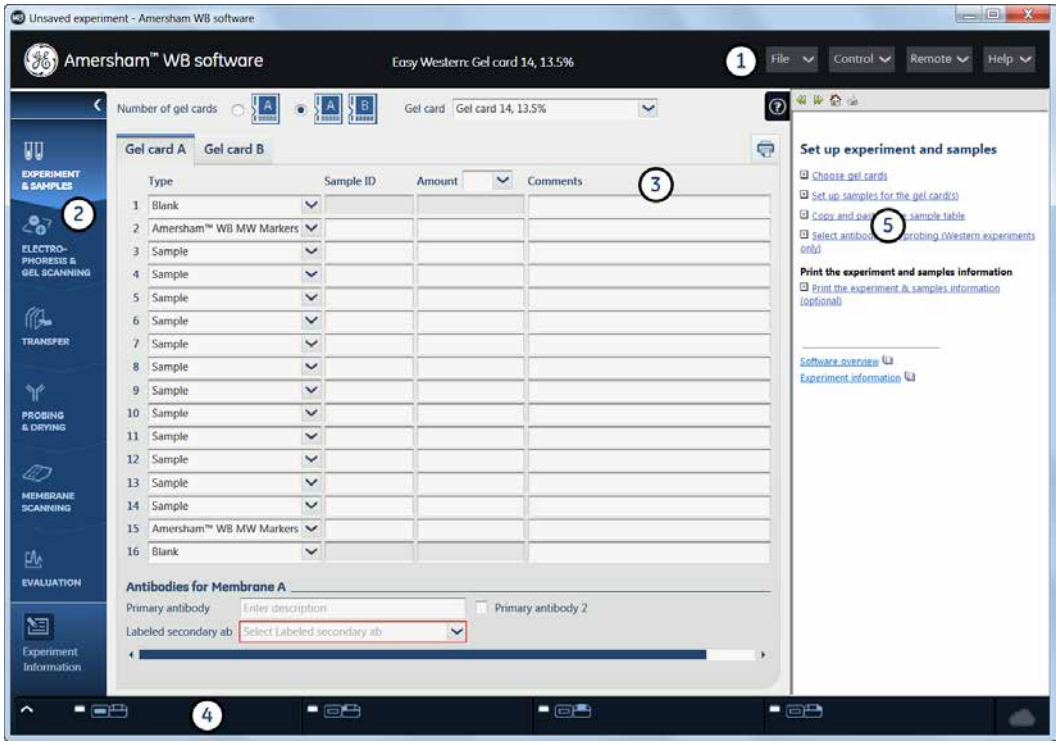
2.9.1 Amersham WB software start screen

In the software start screen it is possible to create new experiments and open previously saved experiments. For information about how to create/open experiments in the software, see *Section 4.3.1 Start the software and create an experiment, on page 111*.



2.9.2 Amersham WB software main screen

The illustration below shows the software main screen for a Western experiment.



Area	Description
1	***Menu bar*** The menu bar consists of four menus: • ***File***: Create, open and save experiments. Print information and exit software. • ***Control***: Abort run, empty transfer and probing chamber, start cleaning of transfer and probing flow paths. • ***Remote***: Connect and disconnect to instrument remote access. Retrieve instrument access codes and manage remote user. Edit the remote connection configuration. ***Note:*** *Only visible when an instrument is connected.* • ***Help***: Generate system reports. Open the help panel, the user manual and the ***About Amersham WB*** dialog. For more information see *Section 2.9.3 Menu bar, on page 67*.

Area	Description
2	***Workflow panel*** The workflow panel shows the different workflow steps in the software for an experiment. It is possible to go forward and backward to view and edit information and parameters in the different workflow steps before starting a run (i.e., electrophoresis, transfer, probing, drying or membrane scanning). It is always possible to go back and edit text in information fields in the different workflow steps during and after a run. For example, the sample table can be filled in after electrophoresis has started. ***Note:*** *For an electrophoresis experiment, the workflow steps **TRANSFER**, **PROBING & DRYING** and **MEMBRANE SCANNING** are dimmed. These steps are only included in Western experiments.* For more information see *Section 2.9.4 Workflow panel, on page 72*.
3	***Work area*** The work area shows the text information, parameters and controls for the currently displayed workflow step.
4	***Instrument status panel*** The instrument status panel shows the instrument connection status. When the instrument is connected the status of each hardware module (electrophoresis and scanning compartment, transfer tank, probing compartment, and drying compartment) and the progress of any run in the modules is shown. Several experiments can be open at the same time. Each experiment requires that a new instance of the software is opened. The instrument status panel will show the status of the instrument for all open experiments. One hardware module can be used by one experiment at a time. This means that four different workflow steps from four different experiments can be run in parallel.
5	***Help panel*** The help panel shows the help instructions for the screen currently displayed in the work area. When selecting a new step in the workflow, the help instructions are automatically updated to display help for the active screen. ***Tip:*** *To view the user manual containing detailed information about the system, select **Help:View user manual** in the menu bar, or press **Ctrl+F1**.* For more information see *Section 2.9.6 Help panel, on page 78*.

2.9.3 Menu bar

File menu

Command	Description
New (Ctrl+N)	Select to open a new instance of the software showing the Amersham WB software start screen. Up to four instances (experiments) of the software can be run on one instrument in parallel.
New from existing	Select to create a new experiment based on an existing experiment. A dialog will open in which to select the experiment to base the new experiment on. A number of settings from the previous experiment will be copied to the new experiment. See *Create a new experiment based on a previous run, on page 117* for more information.
Open (Ctrl+O)	Select to open an existing experiment.
Exit	Select to exit the software.
Save (Ctrl+S)	Click to save the experiment. If you are saving the experiment for the first time, a dialog will appear where you must enter a name for the experiment. ***Save*** is not available when editing an image.
Save as	Click to save the experiment with another name. ***Save as*** is not available during a run or when editing an image.
Print (Ctrl+P)	This selection is only available for the ***EXPERIMENT & SAMPLES*** and ***PROBING & DRYING*** screens. Click to print the experiment and samples information or the probing protocol, depending on which screen is displayed.

Control menu

Command	Description
Empty Transfer tank	Select to empty the transfer tank, for example, if cleaning of the transfer flow path was not performed. ***Note:*** *Make sure that the transfer holders have been removed from the transfer tank before emptying the transfer tank.*
Empty Probing chamber	Select to empty the probing chamber, for example, if cleaning of the probing flow path was not performed. ***Note:*** *Make sure that the membranes have been removed from the probing chamber before emptying the probing chamber.*
Clean Transfer flow path	Select to clean the transfer flow path. Follow the instructions in the dialog that is displayed. ***Note:*** *Make sure that the transfer holders have been removed from the transfer tank before cleaning the transfer flow path.*
Clean Probing flow path	Select to clean the probing flow path. Follow the instructions in the dialog that is displayed. ***Note:*** *Make sure that the membranes have been removed from the probing chamber before cleaning the probing flow path.*

Command	Description
Maintenance clean Transfer flow path	Select to perform a maintenance cleaning of the transfer flow path. See *Maintenance cleaning of transfer flow path, on page 325*. ***Note:*** *Make sure that the transfer holders have been removed from the transfer tank before cleaning the transfer flow path.*
Maintenance clean Probing flow path	Select to perform a maintenance cleaning of the Probing flow path. See *Maintenance cleaning of probing flow path, on page 326*. ***Note:*** *Make sure that the membranes have been removed from the probing chamber before cleaning the probing flow path.*
Abort run	Select to abort an ongoing run. A dialog will open and ask for your confirmation before aborting the run. Click ***OK*** to abort the run.

Remote menu

The ***Remote*** menu is only visible when the computer is connected to an instrument.

Command	Description
Activate/Deactivate remote access	Select to activate/deactivate instrument remote access. When the instrument is activated for the first time, the ***Remote access settings dialog*** opens, where the system name and settings can be edited. See *Appendix A The Amersham WB watch app, on page 363* for more information.
Add WB watch user	Select to receive the instrument access code to be entered in the Amersham WB watch app.

Command	Description
List of WB users	Select to open the ***List of WB users*** dialog to view the remote users that are connected to the instrument. The user ***First name***, ***Last name***, and ***E-mail*** is displayed. It is also possible to remove a user by clicking the ***Remove user*** button.
Remote access settings	Select to edit the system name, and if snapshots of the last images scanned and service information should be uploaded to the cloud. See *Appendix A The Amersham WB watch app, on page 363* for more information.

Help menu

Command	Description
Generate system report	Select to generate a system report. The system report will contain information about the status of the connected instruments as well as of the software installation. It also contains a text field for entering information if a problem has been encountered. When finished the system report is saved in a compressed file by clicking the ***Save system report*** button.
Show/hide help panel (F1)	Select to hide or show the help panel.
View user manual (Ctrl+F1)	Select to view the Amersham WB system User Manual.

Command	Description
About Amersham WB	Select to open the ***About Amersham WB*** dialog. The dialog contains information on: • Amersham WB software version • Elpho & scan unit and Western unit firmware versions • Elpho & scan unit and Western unit serial numbers • trademark and address information (press ***Details*** button) • license agreement (press ***License agreement*** button)

2.9.4 Workflow panel

About the workflow panel

The workflow panel displays the workflow steps associated with the selected experiment.

Panel display

The workflow panel can be minimized or maximized by clicking the arrow,  , in the upper left corner.

In the maximized view the images and names of the workflow steps are displayed. In the minimized view, the images of the workflow steps are displayed.

Navigate between the workflow steps by clicking on the steps or by using the available keyboard shortcuts, see the table below.

A yellow check mark indicates that the workflow step has been completed or, for the ***EXPERIMENT & SAMPLES*** workflow step, all required data has been entered.

Workflow panel overview

Workflow step	Description
EXPERIMENT & SAMPLES (Ctrl + 1)	In this workflow step the user can: • select number of gel cards • select gel card type • enter sample information • enter antibody information See *Section 4.3.2 Set up experiment and samples, on page 119* for more information.

Workflow step	Description
ELECTROPHORESIS & GEL SCANNING (Ctrl + 2)	In this workflow step the user can: • enter gel card information • edit the parameters for the ***Electrophoresis Protocol*** • edit the parameters for the ***Gel Scanning Protocol*** • select automatic or manual ***Scanning Sensitivity*** • start electrophoresis and gel scanning • perform a re-scan of a gel See *Section 4.3.3 Set up electrophoresis and gel scanning protocols, on page 131* for more information.
TRANSFER (Ctrl + 3)	In this workflow step the user can: • enter membrane information • view gel card information • edit solution information • edit the parameters for the ***Transfer Protocol***. • start transfer ***Note:*** *For an electrophoresis experiment, the workflow step **TRANSFER** is dimmed. This step is only included in Western experiments.* See *Section 4.3.4 Set up the transfer parameters, on page 136* for more information.

Workflow step	Description
PROBING & DRYING (Ctrl + 4)	In this workflow step the user can: • view membrane and antibody information • select ***Recover primary antibodies*** • edit solution information • edit probing protocol • start pre-fill of probing chamber • start probing • start drying ***Note:*** *For an electrophoresis experiment, the workflow step **PROBING & DRYING** is dimmed. This step is only included in Western experiments.* See *Section 4.3.5 Set up the probing protocol, on page 139* for more information.
MEMBRANE SCANNING (Ctrl + 5)	In this workflow step the user can: • start membrane scanning • view membrane information • select automatic or manual ***Scanning Sensitivity*** ***Note:*** *For an electrophoresis experiment, the workflow step **MEMBRANE SCANNING** is dimmed. This step is only included in Western experiments.* See *Section 4.3.6 View membrane scanning setup, on page 145* for more information.
EVALUATION (Ctrl + 6)	Evaluate the experiment. See *Chapter 5 Evaluate an experiment, on page 237* for more information.

Workflow step	Description
Experiment Information (Ctrl + I)	Enter or edit experiment information. See *Section 4.3.7 View or enter experiment information, on page 147* for more information.

2.9.5 Instrument status panel

About the instrument status panel

When the instrument is connected, the status of each hardware module (electrophoresis and scanning compartment, transfer tank, probing compartment, and drying compartment) and the progress of any run in the modules is shown in the instrument status bar for all open experiments.

The instrument status panel shows the status for the four modules:

- electrophoresis and scanning in the Elpho & scan unit
- transfer, probing, and drying in the Western unit

Four different workflow steps from four different experiments can be run in parallel. If several experiment workflow steps are run in parallel, the module being used by the displayed experiment will be highlighted blue.

For an ongoing run, the experiment name is shown in the instrument status panel for that workflow step.

To open a running experiment in any instance of the software you can click on the experiment name to display it.

Panel display

The instrument status panel can be minimized/maximized by clicking the arrow  .

In the minimized view the progress bar is displayed.

In the maximized view the progress bar and status message is displayed.

Status messages

Status messages include:

- the name of an experiment, while running. To open the experiment, click the experiment name.
- the instrument connection status
- if a compartment is ready for a new run
- if the run is in progress

- if the run is completed
- if cleaning/maintenance cleaning of the transfer or probing flow path is in progress

---

2.9.6 Help panel

About help panel

Help information for a workflow step in the software is automatically displayed in the help panel on the right side of the screen. Software information and short laboratory work descriptions are provided.

When selecting a new workflow step, the help instructions are automatically updated to show help information connected to the workflow step.

Help panel display

To hide/show the help panel:

- press **F1** on the keyboard

  or

- click the hide/show icon on the top right

To increase/decrease the width of the help panel, drag the help panel list.

Where more detailed information is available for a help instruction step, a link is provided to the *User Manual*.

2.9.7 Keyboard shortcuts

Shortcut	Description
Ctrl + N	Opens a new instance of the software showing the Amersham WB software start screen.
Ctrl + O	Opens an existing experiment.
Ctrl + S	Saves an experiment.
Ctrl + P	Prints the experiment and samples information or the probing protocol, depending on which screen that is displayed.
F1	Opens/closes the help panel in the software.
Ctrl + F1	Opens the *Amersham WB system User Manual*.
Ctrl + 1	Opens the ***EXPERIMENT & SAMPLES*** step in the workflow area.
Ctrl + 2	Opens the ***ELECTROPHORESIS & GEL SCANNING*** step in the workflow area.
Ctrl + 3	Opens the ***TRANSFER*** step in the workflow area.
Ctrl + 4	Opens the ***PROBING & DRYING*** step in the workflow area.
Ctrl + 5	Opens the ***MEMBRANE SCANNING*** step in the workflow area.
Ctrl + 6	Opens the ***EVALUATION*** step in the workflow area.
Ctrl + I	Opens the ***Experiment Information*** dialog.

3 Plan an experiment

Introduction

This chapter describes:

- the two main experiment types: electrophoresis experiments and Western experiments
- Cy5 pre-labeling of proteins
- planning of electrophoresis and Western experiments

In this chapter

This chapter contains the following sections:

3.1 Electrophoresis and Western experiments

Introduction

Two main types of experiments can be performed using Amersham WB system:

- Electrophoresis experiments
- Western experiments

This section briefly describes the different experiment types.

Electrophoresis experiments

In electrophoresis experiments, samples are pre-labeled using Cy5 dye reagent prior to electrophoresis. This enables direct detection of pre-labeled proteins in the sample, eliminating the need for post-staining the gel. In the Evaluation step after gel scanning, automatic analysis of the protein bands is performed and the results are presented in the software.

See *Section 3.2 Pre-labeling of proteins, on page 83* and *Section 3.3 Plan electrophoresis experiments, on page 90* for more information.

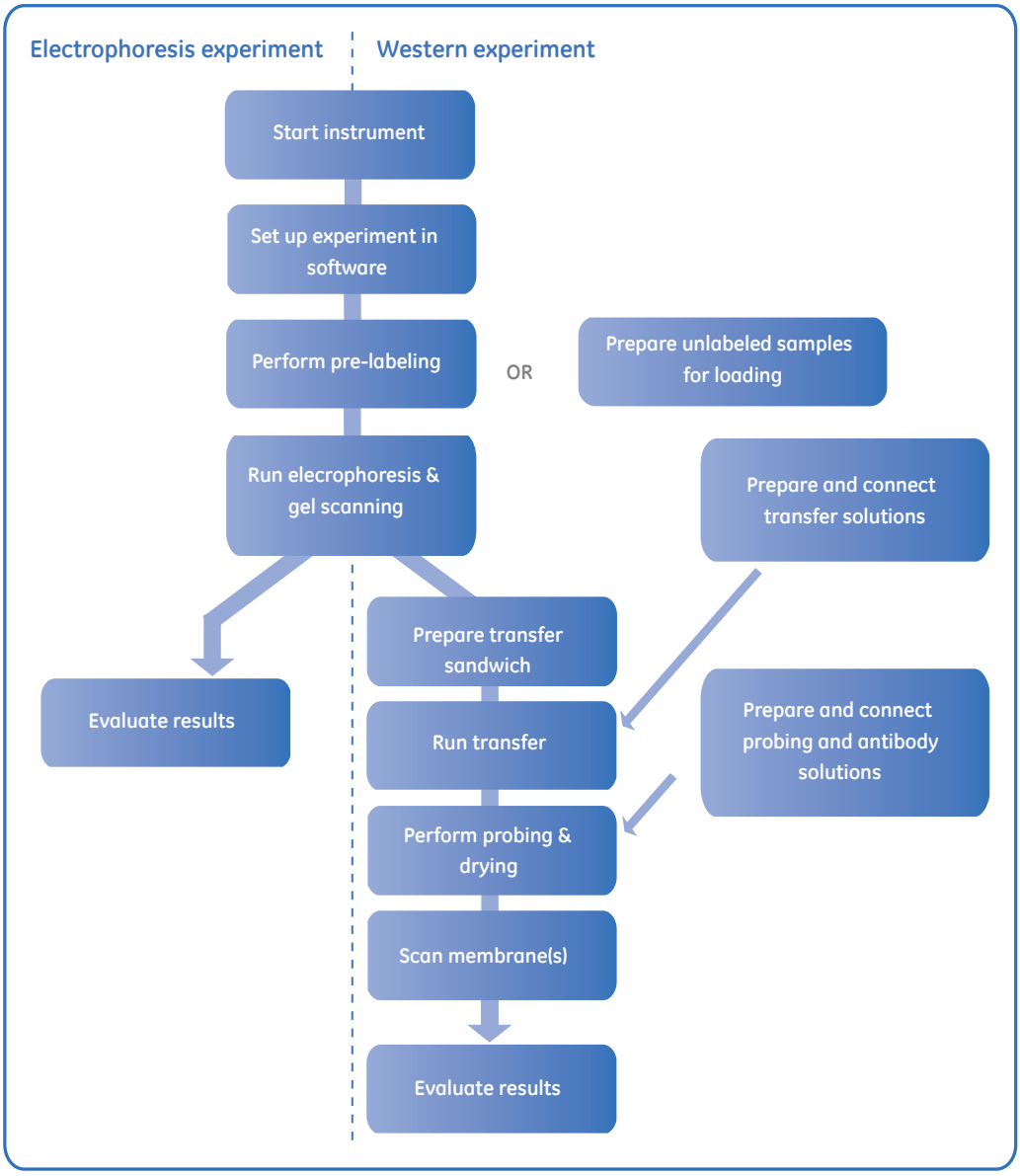
Western experiments

In Western experiments, unlabeled or Cy5 pre-labeled proteins are separated in the gel during electrophoresis and then transferred from the gel to a membrane. The membrane is probed with primary antibodies and secondary antibodies conjugated with Cy3 or Cy5 dye for target protein detection. For total protein detection the sample is pre-labeled with Cy5. After probing, the membrane is dried and then scanned. In the Evaluation step, automatic analysis of the protein bands is performed and the results are presented in the software.

See *Section 3.2 Pre-labeling of proteins, on page 83* and *Section 3.4 Plan Western experiments, on page 93* for more information.

General workflow overview

The illustration below shows the main steps in Electrophoresis and Western experiments.



3.2 Pre-labeling of proteins

Introduction

This section describes Cy5 pre-labeling of proteins for detection in the Amersham WB experiments. It gives information about:

- The protein pre-labeling concept
- Amersham WB Cy5
- Sample types and compatible and interfering buffer substances
- Available labeling protocols

In this section

This section contains the following subsections:

3.2.1 Protein pre-labeling concept

Introduction

This section describes the protein pre-labeling concept in the Amersham WB system. It gives general information about protein pre-labeling, the fluorescent dye reagent, and the labeling reaction.

Pre-labeling of proteins

Pre-labeling of proteins enables protein detection after electrophoresis and Western experiments by automated scanning of gel cards and PVDF cards. The proteins are labeled prior to electrophoresis using the Cy5 dye reagent, enabling detection of protein in the sample, eliminating the need for post-staining the gel or membrane.

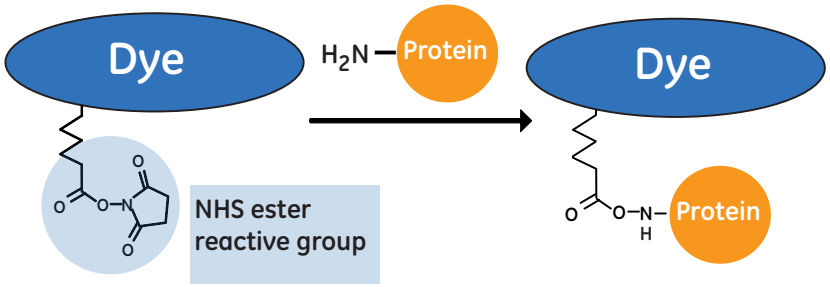
Pre-labeled samples can be used for normalization, in which the total protein signal on the membrane is used as loading control. See *Section 3.4.2 Western normalization strategies, on page 96* for more information.

Cy5 dye reagent

The Cy5 dye reagent has an N-Hydroxy succinimidyl (NHS ester) reactive group, which forms covalent bonds with the epsilon amino group of lysine and the N-terminus of proteins via an amide linkage. Samples with a wide range of protein concentrations (1 ng/µl - 20 µg/µl) can be labeled. Labeling proteins with Cy5 adds approximately 700 to the protein mass. The Cy5 dye reagent is provided in solution (dissolved in DMSO).

Schematic illustration of labeling reaction

The illustration below shows a schematic overview of the labeling reaction.



The table below describes the main steps of the labeling reaction:

Stage	Description
1	The labeling reaction is started by addition of Cy5 dye reagent to the samples.
2	The reaction is stopped by addition of Amersham WB loading buffer, followed by heating.

3.2.2 Pre-labeling pre-requisites and protocols

Introduction

This section describes:

- different sample types
- compatible and interfering substances
- required consumables and products
- protocols

Sample types

When performing pre-labeling of samples, the samples can be divided into three sample types:

- protein samples in compatible buffers
- protein samples in buffer containing interfering substances
- cell lysates or tissue extracts

Depending on the sample type, different pre-labeling procedures are recommended.

Sample concentration

The total protein concentration should be between 1 ng/µl and 20 µg/µl in the original sample. However, the exact protein concentration of the sample does not need to be known.

If samples need to be diluted, it is recommended to dilute protein samples in Amersham WB labeling buffer and cell/tissue samples in their original lysis buffer.

Compatible buffer substances (protein samples)

Buffer salts are compatible in commonly used working concentrations. Additives are compatible in common concentrations or in the indicated concentration ranges.

Examples of buffer substances that are compatible with pre-labeling of protein samples using the SDS-PAGE labeling protocol (described in *Available protocols, on page 89*) are listed below.

Buffer salts	Additives
• Acetate • Bicine • Bis-Tris • Borate • Carbonate • Citrate • HEPES • MES • MOPS • Phosphate (PBS) • PIPPS • Tricine • Tris	• Acetonitrile • Beta-mercaptoethanol (<10 mM) • CHAPS • DTT (<20 mM) • EDTA • Ethanol • Glycerol • $MgCl_2$ • Protease inhibitor mix • SDS (<1%) • Sucrose • TCEP (<20 mM) • Triton X-100 (<1%) • Tween™ (<1%) • Urea

Interfering substances (protein samples)

The sample buffer may contain interfering substances that:

- inhibit the labeling reaction and require buffer exchange

  or

- lower the labeling efficiency

If the sample buffer contains interfering substances that lower the labeling efficiency, then further dilution (default 1:10 in SDS PAGE pre-labeling protocol) of the sample in Labeling buffer, or a buffer exchange using Amersham WB MiniTrap kit into Labeling buffer, may be required to reduce the amount of interfering substances.

Interfering substances requiring buffer exchange

The following substances require a buffer exchange using Amersham WB MiniTrap kit before pre-labeling of samples:

- Imidazole
- Glycine buffers (>100 mM)
- Ammonium sulfate
- Guanidinium-Cl

Interfering substances lowering labeling efficiency

Interference can be reduced or eliminated by dilution or buffer exchange.

Examples of substances that lower the labeling efficiency are listed below:

- Thiols, for example DTT
- Primary amines, for example amino acids and ethanol amine
- Piperazine
- Extreme pH: pH>10, pH<4

Compatible lysis buffers

Common Tris-based lysis buffers with pH 7 to 9, and with various detergents and salts, are compatible with pre-labeling of cell/tissue samples, for example:

- GE mammalian protein extraction buffer
- RIPA buffer

Required consumables and products

The following reagents and solutions are needed for pre-labeling:

- Amersham WB Cy5
- Amersham WB labeling buffer
- Amersham WB loading buffer

See *Section 2.5 Pre-labeling consumables, on page 40* for a description of the pre-labeling consumables components.

If the protein sample buffer contains interfering substances, buffer exchange using the Amersham WB MiniTrap kit may be required. The Amersham WB MiniTrap kit can be ordered separately, see *Chapter 9 Ordering information, on page 360*.

Available protocols

Three protocols are available for pre-labeling of samples: the SDS-PAGE pre-labeling protocol, Western pre-labeling protocol and the Quick SDS-PAGE pre-labeling protocol.

The Quick SDS-PAGE pre-labeling protocol has a decreased incubation time and should only be used for qualitative analysis.

The SDS-PAGE/Quick SDS-PAGE pre-labeling protocols are designed to minimize sample buffer effects on labeling efficiency. A 10 fold dilution of the sample in Amersham WB labeling buffer ensures optimal labeling conditions (see *Compatible buffer substances (protein samples), on page 86*).

The table below summarizes when to use the different protocols:

Protocol	Description	Labeling pH
SDS PAGE pre-labeling protocol	Main protocol for pre-labeling of protein samples. Incubation at room temperature for 30 minutes.	pH 8.5 to 9.0 pH between 8.5 and 9.0 is obtained by 10 times dilution in Amersham WB labeling buffer. If buffer exchange into Labeling buffer has been performed, pH is optimal.
Western pre-labeling protocol	Total protein normalization for cell and tissue samples.	pH 7 to 9
Quick SDS-PAGE pre-labeling protocol	Quick protocol used for pre-labeling of protein samples mainly for qualitative analysis. Incubation at 95°C for 3-5 minutes.	pH 8.5 to 9.0. See SDS PAGE pre-labeling protocol above.

Sample loading capacity

The table below describes the loading capacities of pre-labeled proteins:

Loading volume/well	Protein amount
15-30 µl [1] 20 µl, recommended	Maximum 20 µg/well <0.5 µg/protein band

1 Difference between wells should be <10 µl. Adjust with Amersham WB loading buffer (diluted with an equal volume of ultra pure water).

3.3 Plan electrophoresis experiments

Introduction

This section describes how to plan and set up an electrophoresis experiment.

Select an appropriate gel card type

There are two types of gel cards, homogeneous and gradient gel cards. Choose a suitable gel card type depending on the molecular weight range of your target proteins. Gradient gel cards give a better resolution in the low molecular weight area, and homogeneous gel cards give a better resolution in the high molecular weight area.

Gel card type	Separation resolution, $M_r \times 10^3$
Amersham WB gel card 14, 13.5%, Homogeneous	10 to 225
Amersham WB gel card 14, 8-18%, Gradient	• 6 to 225 (pre-labeled proteins) • 3.5 to 225 (Western experiments, antibody detection)

Reagents for pre-labeling of samples

Pre-labeling of samples is required in an Electrophoresis experiment. Reagents and solutions for pre-labeling of proteins (including Labeling buffer, Cy5 dye reagent and Loading buffer) can be ordered separately. See *Section 3.2 Pre-labeling of proteins, on page 83* for more information.

Amersham WB molecular weight markers

It is recommended to include two MW markers in the run to obtain best possible results in the automatic molecular weight calibration of sample proteins in the image analysis step.

Sample lanes between the two molecular weight marker lanes will use interpolated calibration curves. See *Description of the molecular weight calibration algorithm, on page 315* for more information.

Electrophoresis running buffer

The electrophoresis running buffer for a run is included in the buffer strips.

Electrophoresis experiments overview

There is one experiment type available for Electrophoresis experiments, the Easy SDS-PAGE experiment type.

The table below gives an overview of the different examples of objectives for this experiment type.

For information about automatically calculated results for the experiment type and further analyses that can be performed, see *Chapter 5 Evaluate an experiment, on page 237*.

Objective	Description
Confirmatory analysis	• Confirm presence/absence of a protein • Estimate low/high level of the protein of interest • Confirm molecular weight of sample proteins
Purity check	Estimate target protein:impurity ratio (% purity)
Protein characterization	Protein composition analysis (e.g., molecular weight shift analysis, protein stability, enzymatic cleavage etc.)
LC fractionation analysis	Comparison of different chromatography fractions or pools during protein purification.

Objective	Description
Quantity calibration	Determine amount of target protein in different samples. ***Tip:*** *Use similar buffer conditions for labeling of sample and quantity calibrant.* ***Note:*** *Labeling efficiencies will vary from protein to protein. For quantity measurements, use the same quantity calibrant protein as the sample protein of interest.* ***Note:*** *The software uses linear regression (not forced through the origin) to create the calibration curve.*

3.4 Plan Western experiments

Introduction

This section briefly describes the principles of fluorescent detection and multiplexing and Western normalization strategies in Western experiments in Amersham WB system. It also describes what to consider when setting up an experiment and what solutions are needed for a run.

In this section

This section contains the following subsections:

3.4.1 Fluorescent Western blotting

Introduction

This section briefly describes the principles of fluorescent detection and multiplexing in Amersham WB system.

Multiplex detection

Multiplexed fluorescent Western blotting enables simultaneous detection on the same blot of:

- **two target proteins** (by using Cy3 and Cy5 conjugated secondary antibodies)

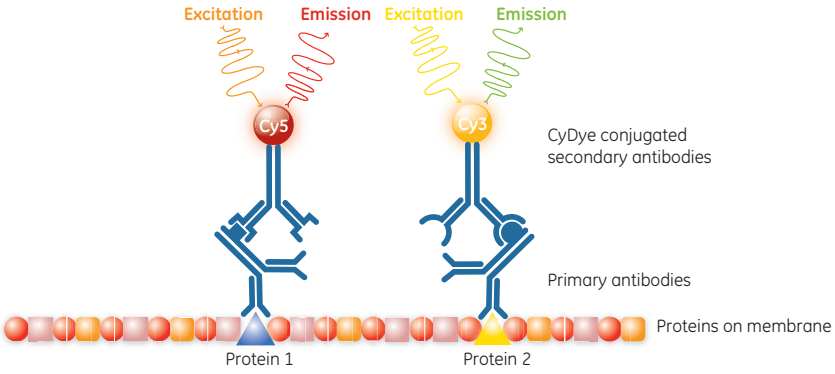
  The target proteins may have the same molecular weight. One of the target proteins can be a housekeeping protein that is used as internal control in Western normalization experiments.

- **Cy5 pre-labeled total protein and one target protein** (by using secondary antibody conjugated to Cy3)

  Cy5 labeled total protein will be transferred to the membrane, since it is a covalent bond between dye and protein. Cy5 pre-labeled total protein can be used as loading control in Western normalization experiments.

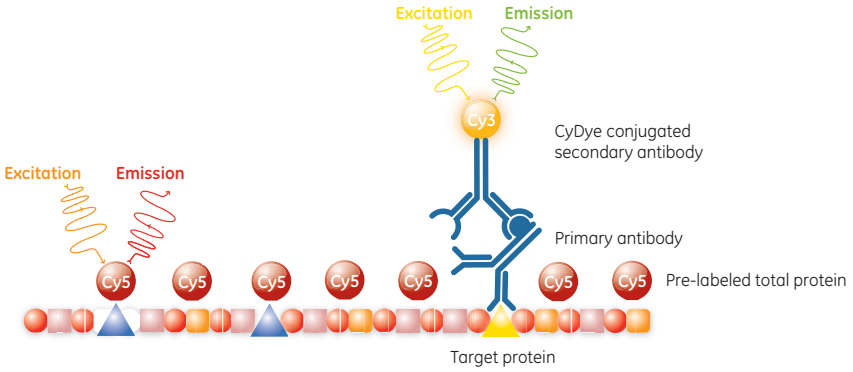
Principle of multiplexing using Cy3 and Cy5 secondary antibodies

The illustration below shows primary antibodies against protein targets on the membrane that are recognized by species-specific Cy3 and Cy5 conjugated secondary antibodies.



Principle of multiplexing by combining Cy5 total protein pre-labeling and Cy3 secondary antibody

The illustration below shows Cy5 total protein labeled cell lysate on the membrane and a target protein detected by a species specific Cy3 conjugated secondary antibody. Cy5 pre-labeled protein is transferred from the gel to the membrane.



3.4.2 Western normalization strategies

Introduction

In this section, principles and differences between the Western normalization strategies for quantitative comparison of target protein levels between samples using Amersham WB system are briefly described.

Normalization for accurate quantitation

To reliably quantitate protein levels in Western experiments between different samples, levels of the protein of interest should be normalized to an internal reference proportional to sample load. Normalization corrects for variations in the amount of total protein loaded between lanes due to errors such as pipetting of sample, incorrect protein quantitation of sample or cell number of sample, that otherwise may result in uneven sample loading.

Normalization options

There are two different Western normalization options for a Western experiment in Amersham WB system:

- Normalization using Cy5 pre-labeled total protein
- Normalization using endogenous or housekeeping protein

Normalization using pre-labeled protein

Total protein labeling of cell lysates or tissue samples can be used as a loading control since the total protein signal is proportional to protein amount. The labeled proteins are transferred and can be detected on the membrane at the same time as a Cy3 antibody against a target protein (see illustration in *Principle of multiplexing by combining Cy5 total protein pre-labeling and Cy3 secondary antibody, on page 95*.

Note: *For linearity of results it is important to use identical labeling conditions (i.e., pH, temperature, volume and dye concentration should not vary between samples).*

Normalization using endogenous protein

If the sample is a cell lysate, an endogenous constitutively expressed "housekeeping" protein (i.e., usually a structural cellular protein which is expressed at a relatively constant rate reflecting cell number) may be used as an internal control.

Commonly used housekeeping proteins for mammalian cells are GAPDH, Tubulin and Actin. The target protein and the housekeeping protein can be detected simultaneously on the membrane by multiplexing using Cy3 and Cy5 secondary antibodies (see illustration in *Principle of multiplexing using Cy3 and Cy5 secondary antibodies, on page 94*).

3.4.3 Experimental setup

Introduction

This section describes what to consider when setting up a Western experiment.

Select an appropriate gel card type

There are two types of gel cards, homogeneous and gradient gel cards. Choose a suitable gel card type depending on the molecular weight range of your target proteins. Gradient gel cards give a better resolution in the low molecular weight area, and homogeneous gel cards give a better resolution in the high molecular weight area.

Gel card type	Separation resolution, $M_r \times 10^3$
Amersham WB gel card 14, 13.5%, Homogeneous	10 to 225
Amersham WB gel card 14, 8-18%, Gradient	• 6 to 225 (pre-labeled proteins) • 3.5 to 225 (Western experiments, antibody detection)

Amersham WB molecular weight markers

- In Western experiments, Amersham WB molecular weight markers should be diluted 1:20 in Loading buffer before loading on the gel card. Before use, the supplied Loading buffer must be diluted with ultra pure water and contain added DTT. For preparation, see *Section 4.4.3 Prepare Amersham WBmolecular weight markers, on page 162*.
- The molecular weight markers can be diluted further in prepared Loading buffer to match samples with weak signals. However, it is then recommended to select the option ***Stop electrophoresis on time***
- It is recommended to include two marker lanes in the run to obtain best possible result in the automatic molecular weight calibration of sample proteins in the image analysis step.

  Sample lanes between the two molecular weight marker lanes will use interpolated calibration curves. See *Description of the molecular weight calibration algorithm, on page 315* for more information.

Pre-labeling of samples

Pre-labeling of samples must be performed in normalized Western experiments where pre-labeled protein is used as a total protein control. See *Section 3.2 Pre-labeling of proteins, on page 83* for more information.

Select primary and secondary antibodies for an experiment

To be able to distinguish between the two targets in a multiplex Western, the primary antibodies must be of two different species and the secondary antibodies need to be species specific and be conjugated with two different CyDyes (Cy3 and Cy5, see illustration in *Principle of multiplexing using Cy3 and Cy5 secondary antibodies, on page 94*).

Cy3 and Cy5 conjugated secondary antibodies directed against both mouse and rabbit primary antibodies are available. Select the appropriate secondary antibody matching the primary antibody species.

Note: *If Cy5 pre-labeling is performed, use Cy3 conjugated secondary antibody for detection of the target protein.*

The table below shows possible combinations of primary and secondary antibodies.

Setup	Primary antibody species	Secondary antibody selections
Detecting one target protein	Mouse	Select one of the secondary antibodies below: • Goat anti-mouse Cy3 • Goat anti-mouse Cy5
	Rabbit	Select one of the secondary antibodies below: • Goat anti-rabbit Cy3 • Goat anti-rabbit Cy5
Detecting two target proteins	One mouse and one rabbit	Select one of the secondary antibody combinations below: • Goat anti-mouse Cy3 and Goat anti-rabbit Cy5 **or** • Goat anti-mouse Cy5 and Goat anti-rabbit Cy3

Setup	Primary antibody species	Secondary antibody selections
Detecting Cy5 pre-labeled protein and one target protein	Mouse	Goat anti-mouse Cy3
	Rabbit	Goat anti-rabbit Cy3
Detecting more than two target bands that differ in molecular weight	Mouse and/or rabbit	Appropriate mix of secondary antibodies ***Note:*** *Pre-run individual antibodies separately to determine what bands each antibody detects.*

Probing time

Optimal probing time varies between different primary antibodies from approximately 15 minutes (high abundant target or high affinity of antibody) to overnight (low abundant or low affinity). 1 hour probing time is sufficient for most primary antibodies and is set as default, but for low abundant targets it is recommended to optimize/prolong the probing time for best results.

The secondary antibody probing time is optimized to 30 minutes (set as default) and works for targeting most primary antibodies.

3.4.4 Western experiments setup overview

Introduction

This section gives an overview of the Western experiment types.

Western experiments overview

The table below gives an overview of the different Western experiment types and examples of objectives for an experiment type.

For information about automatically calculated results for the experiment types and further analyses that can be performed, see *Chapter 5 Evaluate an experiment, on page 237*.

Experiment type	Example of objectives
Easy Western	• Confirm presence/absence of up to two proteins • Estimate low/high level of the target protein(s) • Confirm molecular weight of target protein
Western with total protein normalization	Compare normalized target protein levels between samples. *For example:* Study the effect of various treatments and conditions on target protein levels.
Western with endogenous protein normalization	Compare normalized target protein levels between samples. *For example:* Study the effect of various treatments and conditions on target protein levels.

3.4.5 Western experiment buffers and solutions

Introduction

This section describes the recommended secondary antibody dilutions and how to determine optimum antibody concentrations. It also lists the buffers and solutions that need to be prepared for a Western experiment.

Recommended dilution for secondary antibodies

The recommended dilution for secondary antibodies (1 µg/µl) is 1:2500, but the optimal dilution (between 1:2500 to 1:4000) should be determined for each Western experiment setup. See below for information about how to optimize the primary and secondary antibody dilution.

Primary and secondary antibody optimization

Note: *The signal-to-noise ratio is not the only parameter to look at. The linearity of the response is equally important for normalization purposes. This is valid for both primary and secondary antibodies.*

Step	Action
1	Make a serial dilution of the protein that the primary antibody is directed against.
2	Perform full Western blotting procedure.
3	Block the membranes.
4	Wash and incubate the membranes with several dilutions of the primary antibody, for example, 1:100, 1:500, 1:1000 and 1:1500.
5	Wash and incubate the membranes with the secondary antibody, using the same dilutions that were used for the primary antibody.
6	Select the optimal antibody dilution as the maximal signal-to-noise ratio.
7	Make a serial dilution of the protein that the primary antibody is directed against.
8	Perform full Western blotting procedure.

Step	Action
9	Block the membranes.
10	Wash and incubate the membranes with the optimal dilution of the primary antibody.
11	Wash and incubate the membranes with several dilutions of the secondary antibody, for example, 1:1250, 1:1500, 1:3500 and 1:4000.
12	Select the optimal antibody dilution for maximal signal-to-noise ratio with linear and proportional response.

Transfer solution recipes

WARNING

Place the Amersham WB analyzer in a room with exhaust ventilation if methanol or other chemicals that need ventilation will be used.

CAUTION

Fire hazard. Do not use transfer buffer solutions consisting of more than 40% ethanol or 40% methanol. It is recommended to only use 20% ethanol in transfer buffers.

Solution	Recipe	Amount/run
Transfer buffer	25 mM Tris, 192 mM glycine, 20% ethanol pH 8.3 It is possible to use methanol instead of ethanol in the transfer buffer. ***Note:*** *Some ethanols have auto fluorescent properties which will lead to a high membrane background. Make sure that the ethanol used during transfer is not auto fluorescent.*	1000 ml
Ultra pure water (used for cleaning)	Ultra pure water	1000 ml

Probing and antibody solution recipes

Solution	Recipe	Amount/run
Blocking solution	The system is compatible with most blocking solutions, for example 2% ECL PRIME agent in PBS-T (0.1% Tween™-20 in 1×PBS), or 5% Bovine Serum Albumin in PBS. Use blocking solutions with low auto fluorescence, see *Western Blotting – Principles and Methods* handbook for recommendations.	70 ml
Wash buffer	PBS-T (0.1% Tween-20 in 1×PBS)	560 ml
Final wash buffer	PBS pH 7.4	290 ml
Antibody solution	Antibodies in PBS-T (0.1% Tween-20 in 1×PBS). See *Primary and secondary antibody optimization, on page 102* for how to determine optimum antibody concentrations.	5 to 12 ml
Ultra pure water (used for rinsing between probing steps and cleaning of probing flow path)	Ultra pure water	760 ml

4 Perform an experiment

About this chapter

Electrophoresis (gel electrophoresis and scanning) experiments and Western experiments (gel electrophoresis and Western blot steps) can be performed using Amersham WB system. This chapter describes how to set up and run an experiment.

For information about evaluation of experiments, see *Chapter 5 Evaluate an experiment, on page 237*.

For information about workflows and background information for pre-labeling of samples, electrophoresis and Western experiments, see *Chapter 3 Plan an experiment, on page 80*.

In this chapter

This chapter contains the following sections:

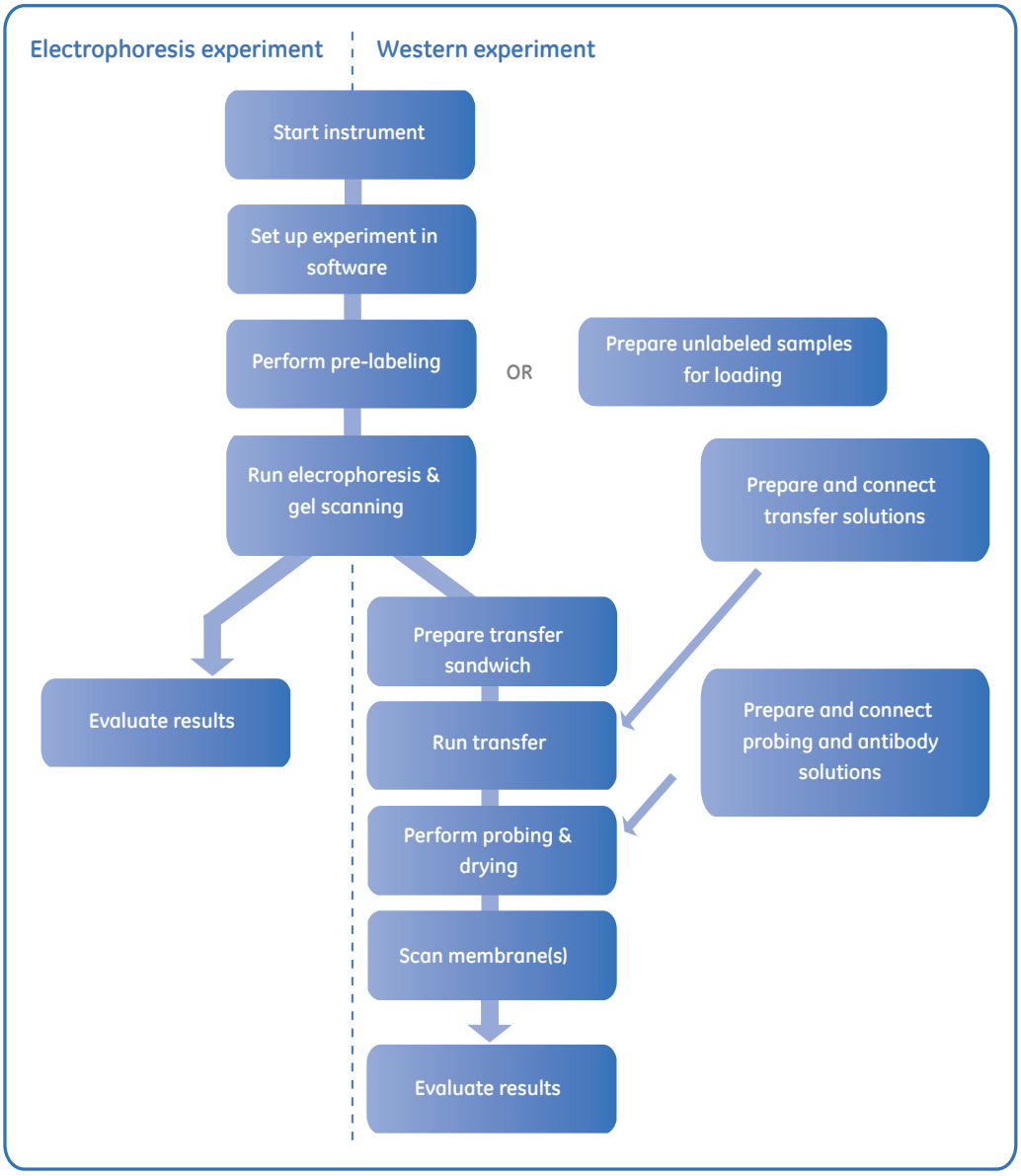
4.1 Overview

Introduction

This section gives a workflow overview for Electrophoresis and Western experiments. For more information about the different types of experiments and pre-labeling of samples, see *Chapter 3 Plan an experiment, on page 80*.

General workflow overview

The illustration below shows the main steps in Electrophoresis and Western experiments.



4.2 Start the Amersham WB analyzer

Introduction

This section describes how to start up the Amersham WB analyzer units. It is possible to set up an experiment in advance on an office computer with no connection to the instrument.

Note: *Both instrument units must always be turned on during a run.*

Start the Amersham WB elpho & scan unit

Step	Action
1	On the rear panel of the Elpho & scan unit, press the mains power switch to **I** (ON).  *Result:* The Elpho & scan unit is started.
2	During start-up, the indicator panel displays the following:  When the instrument unit is ready to use, a white square is displayed. 

Start the Amersham WB western unit

Step	Action
1	On the rear panel of the Western unit, press the mains power switch to **I** (ON). ***Note:*** *The Elpho & scan unit must be turned on before it is possible to run the Western unit.*  *Result:* The Western unit is started.
2	During start-up, the indicator panel displays the following:  When the Western unit compartments are ready to use, a white square is displayed for each of the three compartments (transfer, probing and drying). 

4.3 Set up an experiment in Amersham WB software

Introduction

This section describes how to start the Amersham WB software and how to create, set up and save an experiment in the software.

Help instructions for each workflow step are also available in the *Amersham WB software help* in the right software panel.

In this section

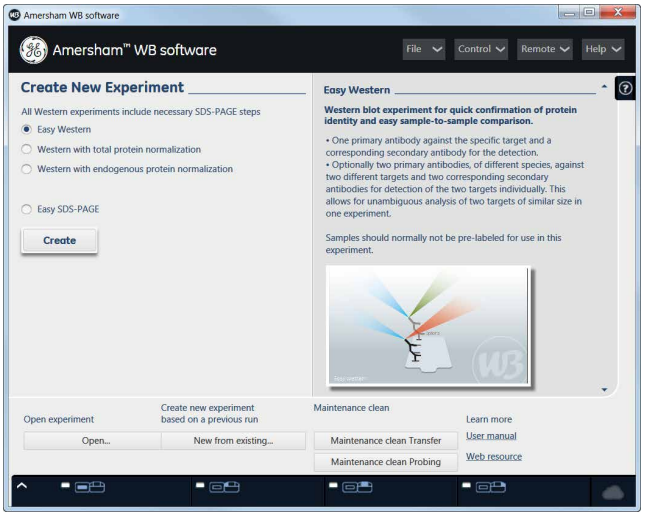
This section contains the following subsections:

4.3.1 Start the software and create an experiment

Start the software

Step	Action
1	Start the computer and log on to Microsoft Windows.
2	Double-click the Amersham WB software icon on the desktop.

Result: The software start screen is displayed.



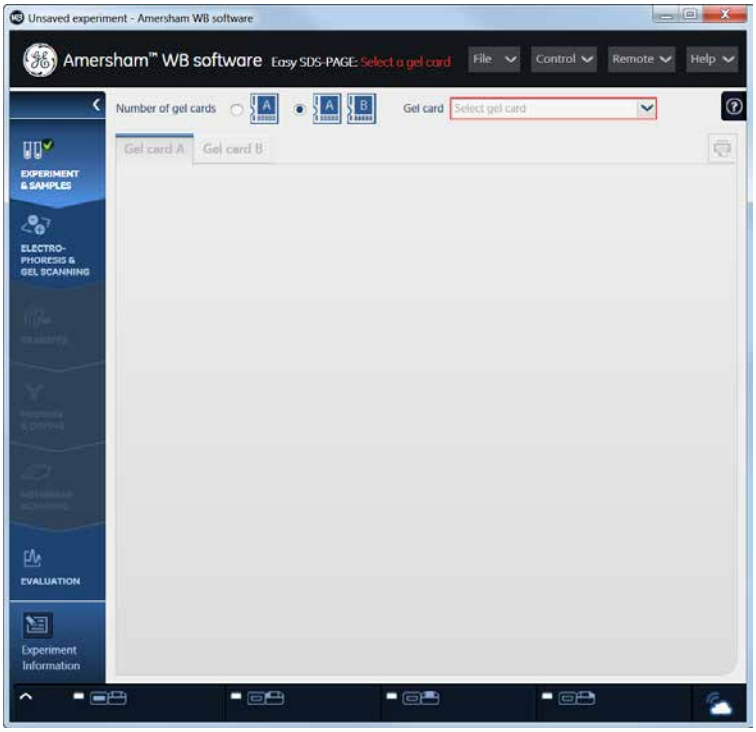
Tip: *To show the help panel with help instructions for the active screen, click the help panel button*  *or press* ***F1****.*
To open the user manual, select ***Help:View user manual*** *or press* ***Ctrl + F1****.*

Create a new Electrophoresis experiment

Step	Action
1	In the ***Amersham WB software*** start screen, select the ***Easy SDS-PAGE*** experiment type in the ***Create New Experiment*** area.

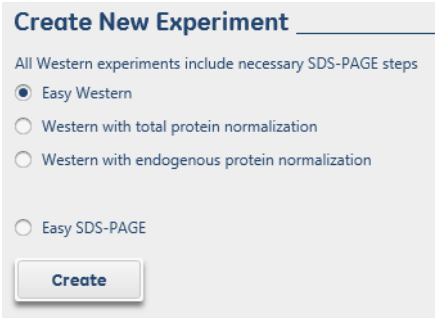
Result: A description of the experiment type is displayed on the right side of the start screen.

For further information about examples of objectives within this experiment type, see *Electrophoresis experiments overview, on page 91*.

Step	Action
2	Click ***Create***. *Result:* The Amersham WB software main screen opens displaying the ***EXPERIMENT & SAMPLES*** workflow step. The workflow steps associated with electrophoresis and gel scanning and evaluation are enabled in the workflow panel. For a description of the different areas in the main screen, see *Section 2.9.2 Amersham WB software main screen, on page 65*.  Continue with the instructions in *Section 4.3.2 Set up experiment and samples, on page 119*.

Create a new Western experiment

Step	Action
1	In the ***Amersham WB software*** start screen, select the appropriate Western experiment type in the ***Create New Experiment*** area.

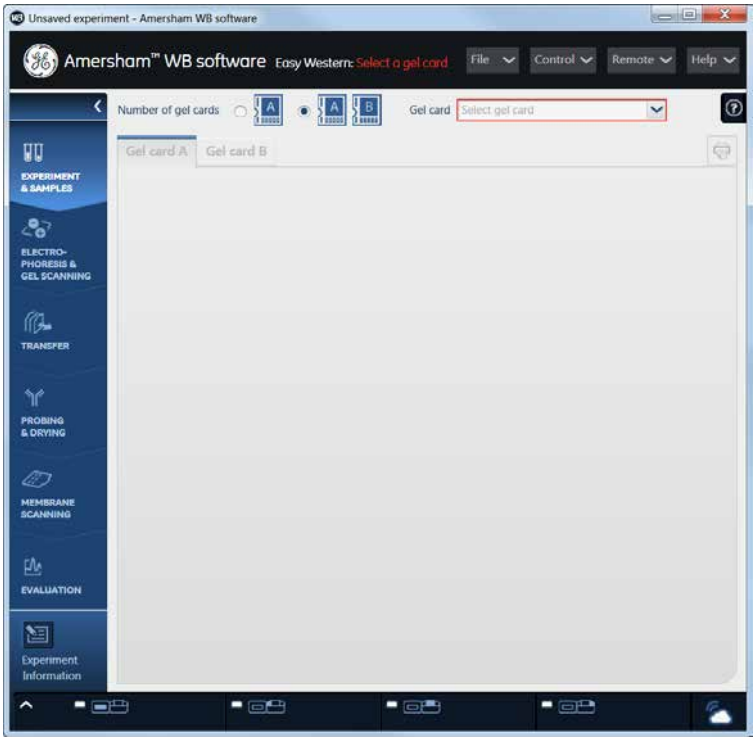


Result: A description of the selected experiment type is displayed on the right side of the software start screen.

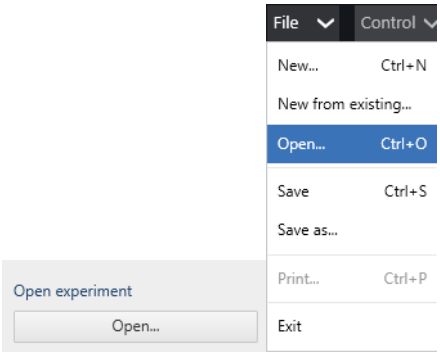
Note:

Make sure to select the correct experiment type when creating a new experiment. It is not possible to edit the experiment type within the experiment once created.

For further information about the experiment types, see *Section 3.4.4 Western experiments setup overview, on page 101*.

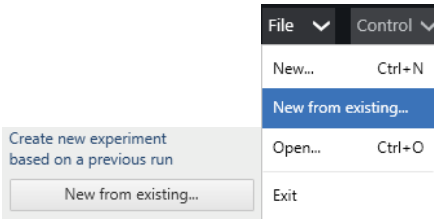
Step	Action
2	Click ***Create***. *Result:* The Amersham WB software main screen opens displaying the ***EXPERIMENT & SAMPLES*** workflow step. The workflow steps associated with electrophoresis and gel scanning, transfer, probing & drying, membrane scanning and evaluation are enabled in the workflow panel. For a description of the different areas in the main screen, see *Section 2.9.2 Amersham WB software main screen, on page 65*.  Continue with the instructions in *Section 4.3.2 Set up experiment and samples, on page 119*.

Open a previously saved experiment

Step	Action
1	Click the ***Open...*** button or select ***File:Open...*** (Ctrl + O) in the menu bar in the ***Amersham WB software*** start screen.  *Result:* The ***Open*** dialog opens.
2	Browse and select the appropriate Amersham WB experiment file (*.AWBexp) and click ***Open***. *Result:* The selected Amersham WB experiment file opens up in the main screen and: • the ***EXPERIMENT & SAMPLES*** screen is displayed or • if all workflow steps have been run, the ***EVALUATION*** workflow step is displayed

Create a new experiment based on a previous run

Step	Action
1	Click the ***New from existing...*** button or select ***File:New from existing...*** in the menu bar in the ***Amersham WB software*** start screen.



Result: The ***Open*** dialog opens.

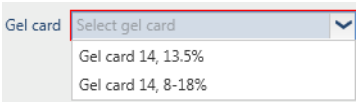
Step	Action
2	Browse and select the appropriate Amersham WB experiment file (*.AWBexp) and click ***Open***. *Result*: The selected Amersham WB experiment file opens up in the main screen and the ***EXPERIMENT & SAMPLES*** workflow step is displayed. The following information in the selected experiment is transferred to the new experiment that is created: • ***EXPERIMENT & SAMPLES***: - Experiment type (cannot be edited) - Gel card type - Sample type - Amount (only for sample types ***Quantity calibrant***) - Unit (for Amount) - Secondary antibody type - Primary antibody name • ***ELECTROPHORESIS & GEL IMAGING***, ***TRANSFER***, ***PROBING & DRYING***: Any default settings that have been edited are transferred. • ***EVALUATION***: All settings for the different image positions in the experiment are transferred. The actual images and associated data are not transferred. • ***Experiment Information***: Information entered about consumables (***Consumable type***) in the ***Cards & reagents*** tab is transferred. ***Note:*** *Experiment specific data such as consumable ID, batch data and general comments about the experiment are not transferred.*
3	Continue with the instructions in *Section 4.3.2 Set up experiment and samples, on page 119*.

4.3.2 Set up experiment and samples

Choose gel cards

Step	Action
1	Choose the number of gel cards for the experiment in the ***Number of Gel cards*** area in the ***EXPERIMENT & SAMPLES*** screen. If one gel card is included, position A must be used. 

Step	Action
2	Select the type of gel card to be used in the ***Gel card*** drop-down list.

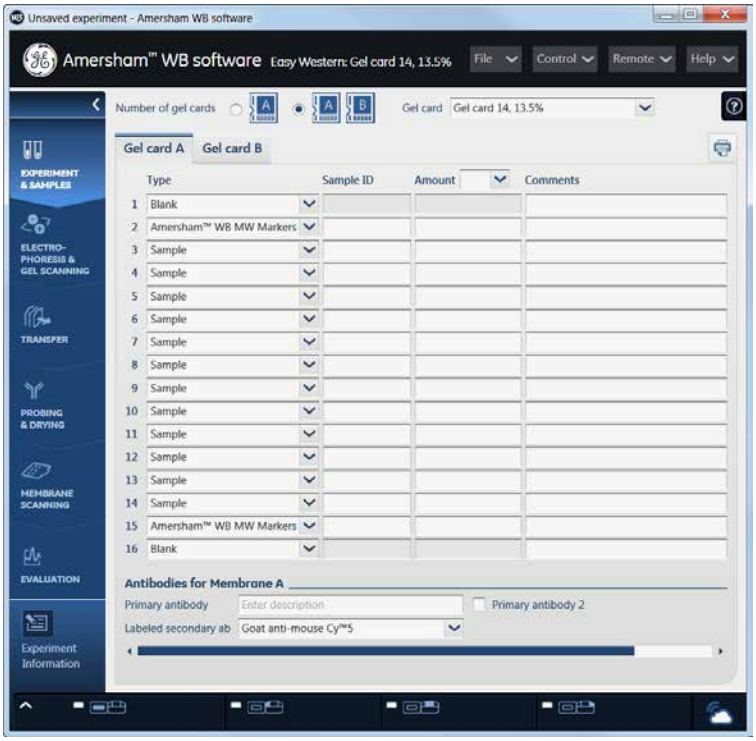


See *Section 3.3 Plan electrophoresis experiments, on page 90* for information of the separation resolution for the different types of gel cards

Note:

The A and B gel cards must be of the same type.

Result: The ***Gel Card A*** tab displays the sample table where sample information can be entered.



Sample table overview

On the ***Gel card A*** tab (and ***Gel card B*** tab if included in the experiment), the sample table is displayed. Each row in the table corresponds to a well in the gel card.

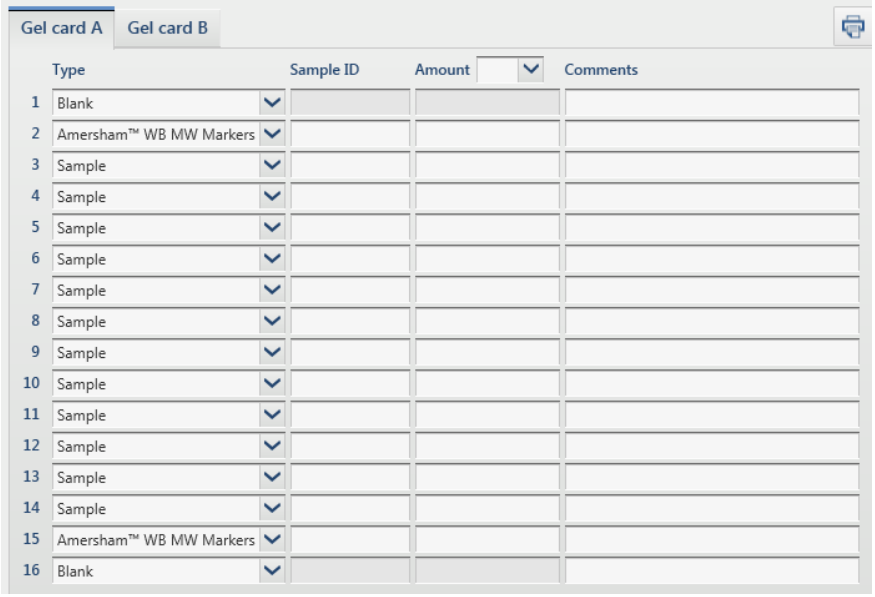
By default:

- the first and last well on the gel card (in the ***Type*** column of the table) are set to ***Blank***,

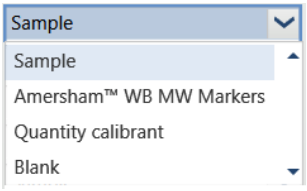
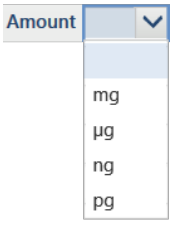
  Note: *It is recommended to load lane 1 and 16 with Loading buffer to optimize the performance and minimize the potential outer lane artifacts.*

- the second and the fifteenth well are set to ***Amersham™ WB MW markers***,
- the other wells are set to ***Sample***.

If you are performing a quantity calibration experiment, the samples to be used as calibrants, should be set to type ***Quantity calibrant***.



Set up the samples for the gel card(s)

Step	Action
1	To change the type of sample for a well, select the drop-down arrow for the well in the ***Type*** column and select the appropriate sample type.  See *Sample table – Type column options, on page 124* for a description of the sample types and when to use a sample type.
2	**For Quantity calibration experiments only** (otherwise proceed with step 3) ***Note:*** *For information about when to use the quantity calibration sample type, see Sample table – Type column options, on page 124 below.* To set samples to quantity calibrants and enter amounts: • In the ***Amount*** column header, click the drop-down arrow and select the appropriate unit.  • For each well containing a quantity calibrant: - In the ***Type*** column drop-list, select ***Quantity calibrant***. *Result:* The corresponding ***Amount*** field is indicated with a red frame. - Click in the ***Amount*** field with a red frame and enter the protein amount.

Step	Action
3	Enter sample ID and comments for a sample (optional). For each well, click in the appropriate: • ***Sample ID*** field and enter an ID for the sample • ***Comments*** field and enter a comment, for example, sample concentration, sample volume, buffer used etc. ***Tip:*** *Sample IDs and Comments can be entered at any time, also during or after a run.* ***Tip:*** *It is possible to add general comments about the experiment at any time by clicking the* ***Experiment Information*** *icon in the workflow panel.*  *See Section 4.3.7 View or enter experiment information, on page 147 for more information.*
4	Repeat the steps for ***Gel card B*** if included in the experiment. ***Tip:*** *It is possible to copy and paste the whole sample table or parts of the sample table. See Copy and paste in the sample table, on page 125.*

Sample table – Type column options

Type	Description & when to use
Sample	Use this type for a sample to be investigated.
Amersham™ WB MW markers	One or several lanes can be used for Amersham WB molecular weight markers. Make sure that the lanes containing the MW markers are set to ***Type: Amersham™ WB MW markers***. When a new experiment is set up the second and the fifteenth well are set to ***Amersham™ WB MW markers*** by default. The lanes set to ***Amersham™ WB MW markers*** are used by the software to automatically calculate the molecular weight calibration curves. Then, by using the calibration curves, the molecular weights of all detected bands on the image are automatically calculated. See *Description of the molecular weight calibration algorithm, on page 315* for information about how the molecular weight calibration curve is calculated.
Quantity calibrant	In quantity calibration (i.e., an experiment where the amount of target protein in different samples should be determined using a calibration curve), the sample type ***Quantity calibrant*** must be used for the calibrants. This sample type is available for quantity calibration in ***Easy SDS-PAGE*** and ***Easy Western*** experiments. The lanes set to ***Type: Quantity calibrant*** are used by the software to automatically calculate the calibration curve. Then, by using the calibration curve, the absolute protein amount for the samples are automatically calculated.
Blank	Generally used as default type for the outer lanes where the wells are loaded with Amersham WB loading buffer (diluted with an equal volume of ultra pure water). This sample type can also be used for any other lanes loaded with Loading buffer.

Copy and paste in the sample table

It is possible to copy the whole sample table or cells of the sample table and paste into the sample table on another tab (e.g., from ***Gel Card A*** tab to ***Gel card B*** tab) or into, for example, a Microsoft Excel® sheet.

Step	Action
1	To select cells in the sample table: • To select one cell in the sample table: Click in the cell. • To select a number of cells: Click in a cell, hold down the left mouse button and drag a rectangle to include the cells to be copied/pasted. • To select the whole sample table for copy/paste: Click in a cell in the sample table and press **Ctrl + A**. It is also possible to click on ***1*** in the upper left corner of the sample table (representing well number 1), hold down the left mouse button and drag a rectangle to include all cells in the sample table. You do not need to select the column headers. *Result:* Selected cells are highlighted in blue.
2	To copy the selected cells: • press **Ctrl + C** on the keyboard, or • right-click on the sample table and select ***Copy***.
3	To paste the selected cells into the sample table for another position: • Click the cell which corresponds to the top left cell of your selection and press **Ctrl + V** on the keyboard. or • Right-click the cell which corresponds to the top left cell of your selection and select ***Paste***. *Result:* The cells are pasted into the sample table.

Step	Action
4	To paste the selected cells into, for example, Microsoft Excel: Click a cell in an Excel sheet and press **Ctrl+V** on the keyboard or use the paste control in the application. *Result:* The selected cells and the table columns of the sample table are pasted into the work sheet.

Select antibodies for probing (Western experiments only)

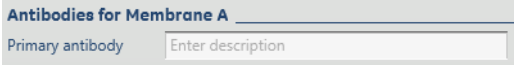
Depending on the type of Western experiment, one or two sets of antibodies can be entered in the ***Antibodies for Membrane A*** (or B) area.

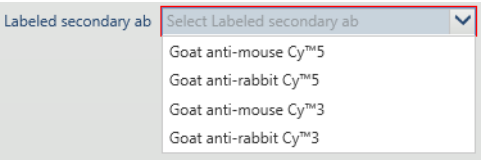
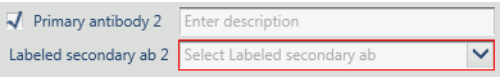
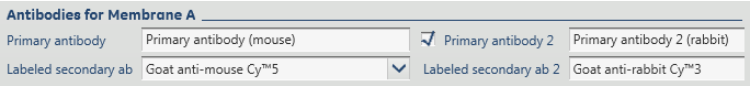
Antibodies for probing of membranes in positions A and B are entered under the gel card A and B tabs. The type of secondary antibody to be used needs to be entered before probing can be started. If there are two primary antibodies used, the selection in the gel card tab will determine which antibody is displayed as target and which is displayed as control in further analysis. The first selected primary antibody will be shown in the membrane target image and the second selected primary antibody will be shown in the membrane control image.

See *Select primary and secondary antibodies for an experiment, on page 99* for information of how to combine antibodies.

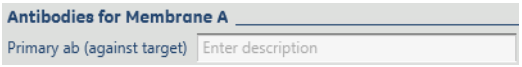
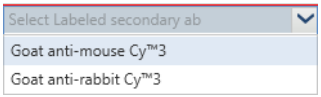
Select antibodies for Easy Western experiments

In ***Easy Western*** experiments, one or two different target proteins can be detected. To enter information:

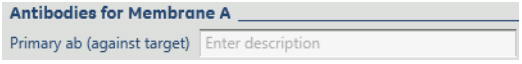
Step	Action
1	Enter a description for the primary antibody in the ***Primary antibody*** field (optional). 

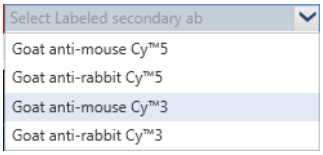
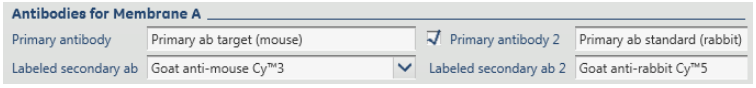
Step	Action
2	Select the appropriate secondary antibody from the ***Labeled secondary ab*** drop-down list. ***Note:*** *The secondary antibody must match the primary antibody species (if the primary antibody species is mouse, the secondary antibody must be of type anti-mouse).* 
3	If a second target protein should be detected check the ***Primary antibody 2*** box. *Result:* Fields for the antibodies to be used for detecting a second target protein are displayed. 
4	Type in a description for the second primary antibody in the ***Primary antibody 2*** field (optional). ***Note:*** *The **Primary antibody 2** species should be a different species than the **Primary antibody** species.*
5	Select the appropriate secondary antibody 2 from the ***Labeled secondary ab 2*** drop-down list. ***Note:*** *The **Labeled secondary ab 2** must match the **Primary antibody 2** species and have another CyDye conjugated than the **Labeled secondary ab**.* **Example:** 

Select antibodies for Western with total protein normalization experiments

Step	Action
1	Type in a description for the primary antibody in the ***Primary ab (against target)*** field (optional). 
2	Select the appropriate secondary antibody from the ***Labeled Secondary ab*** drop-down list (must match the primary antibody species).  ***Note:*** *In this case Cy5 has been used for pre-labeling, so only secondary antibodies conjugated to Cy3 are available.*

Select antibodies for Western with endogenous protein normalization experiments

Step	Action
1	Type in a description for the primary antibody in the ***Primary ab (against target)*** field (optional). 

Step	Action
2	Select the appropriate secondary antibody from the ***Labeled secondary ab*** drop-down list. ***Note:*** *The secondary antibody must match the primary antibody species (if the primary antibody species is mouse, the secondary antibody must be of type anti-mouse).* 
3	Type in a description for the second primary antibody in the ***Primary ab (against control)*** field (optional). ***Note:*** *The **Primary ab (against control)** species should be a different species than the **Primary antibody (against target)** species.*
4	Select the appropriate secondary antibody 2 from the ***Labeled secondary ab 2*** drop-down list ***Note:*** *The **Labeled secondary ab 2** must match the **Primary antibody (against control)** species and have another CyDye conjugated than the **Labeled secondary ab**.* **Example:** 

Print the experiment & samples information (optional)

To print the entered experiment and samples information, click the ***Print*** icon to the right of the gel card tabs, or select ***File:Print***, or press **Ctrl + P**. Select printer in the ***Print*** dialog and print out the information.

The following information will be printed:

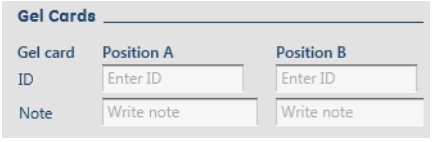
- experiment name
- the gel card type used in the experiment
- the sample tables for gel card A and B
- the antibodies to be used for probing (Western experiments only)

4.3.3 Set up electrophoresis and gel scanning protocols

Introduction

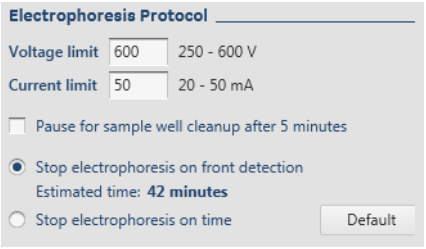
This section describes the electrophoresis and gel scanning parameters.

Enter gel card information

Step	Action
1	To enter the ID of a gel card: In the ***ELECTROPHORESIS & GEL SCANNING*** screen, place the cursor in the ***ID*** field for ***Position A*** and: • scan the data matrix code on the gel card using the data matrix tag reader **or** • enter the ID manually (five digits)  ***Tip:*** *Entering ID should be done just before the gel card is placed on the card plate in the loader.* *If a data matrix tag reader is a used, Code number and Lot number of the gel card will be logged in the* ***Experiment Information*** *dialog.*
2	To enter a note about a gel card, place the cursor in the ***Note*** field for ***Position A*** and type in a note. More information can be added in the ***Experiment Information*** dialog. ***Note:*** *A note can also be written in the writing surface of the physical gel card. Use a pencil, not a pen.*
3	Repeat the steps above for ***Position B***, if included in the experiment.

View/edit the electrophoresis protocol

Note: *The default settings for the **Electrophoresis Protocol** do not normally need to be edited. By default, the electrophoresis is stopped using front detection, that is, when the front has reached the end of the gel.*



If you need to edit any settings in the ***Electrophoresis Protocol*** area, the table below gives a description of the settings. To revert to default settings and values click the ***Default*** button.

Command	Description
Voltage limit [1]	Shows the maximum voltage to be used during the run. Decreasing this value will extend the run time.
Current limit [1]	Shows the maximum current to be used during a run. Decreasing this value will extend the run time.
Pause for sample well cleanup after X minutes [2]	Check this box to pause the electrophoresis after the calculated time in minutes and perform sample well cleanup. The sample well cleanup is performed for pre-labeled samples by dipping a paper comb in the sample wells to remove excess dye after the proteins have migrated into the gel.

Command	Description
Stop electrophoresis on front detection	The electrophoresis run is stopped when the front has reached the end of the gel (i.e., right before the buffer strip opening on the gel card). An ***Estimated time*** for the run is shown only when using default current and voltage limits. This radio button is selected by default. ***Note:*** *If performing a Western experiment where no molecular weight markers are included and pre-labeling of samples is not performed, the electrophoresis can not be stopped using front detection. Use the option* ***Stop electrophoresis on time*** *instead.* ***Note:*** *If the molecular weight marker dilution is higher then 20 times, it is recommended to* ***Stop electrophoresis on time*** .
Stop electrophoresis on time	Select this radio button to stop the run after a specified time. Enter the run time for the electrophoresis run in the ***Stop electrophoresis on time*** field. The run will be stopped when the set time has passed, regardless of the position of the front. ***Tip:*** *If you want to increase the separation of larger proteins in the upper area of the gel card and know what run time is suitable, it is possible to enter a duration time for the run in the protocol to determine the length of the run instead of stopping it based on front detection. The front and smaller proteins may then migrate through and exit the gel.*

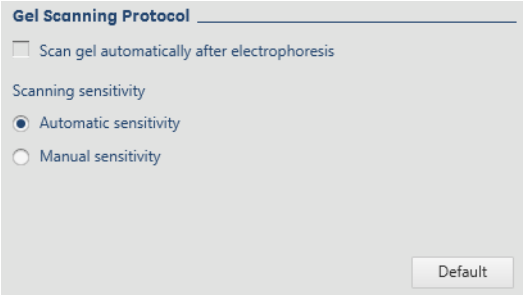
Command	Description
Default	Click to revert to default parameters. A dialog asking for your confirmation will be displayed.

1 Maximum power is 20 W/gel card. The maximum values for the voltage and current parameters can not be reached simultaneously.

2 The time will be calculated from the set current limit and voltage limit. The default limits will set the time to 5 minutes.

View/edit the gel scanning protocol

Note: *The default settings for gel scanning is adjusted to the experiment type to be run. These settings do not normally need to be edited.*



If you need to edit any settings in the ***Gel Scanning Protocol*** area, the table below gives a description of the settings. To revert to default settings and values click the ***Default*** button.

Command	Description
Scan gel automatically after electrophoresis	When this box is checked, the gel cards are automatically scanned after the electrophoresis run. This box is checked by default for the applications that requires pre-labeling of samples, that is ***Easy SDS-PAGE*** and ***Western with total protein normalization***. The status of the box can be altered for all experiments, except for ***Easy SDS-PAGE*** where the gel is always scanned after the electrophoresis run.

Command	Description
Scanning sensitivity	
Automatic sensitivity	Select this radio button to use the automatic sensitivity function at gel card scanning. The scanner and software automatically optimize the scanning sensitivity level for the gel images. The sensitivity level is set so that the strongest protein signals in the gel image do not become saturated (the front and the outermost lanes are not used when sensitivity is optimized). This radio button is selected by default.
Manual sensitivity	Select this button to set/change the sensitivity level of the gel scanning manually. For example: • if you get weak signals for your proteins of interest, try a higher sensitivity level and re-scan the gel cards • if you get saturated signals for your proteins of interest (indicated by a red color in the image preview), try a lower sensitivity level and re-scan the gel cards Change the sensitivity level by dragging the ***Cy5 sensitivity level*** slider.
Default	Click to revert to default settings and values set in the software. A dialog asking for your confirmation will be displayed.

4.3.4 Set up the transfer parameters

Introduction

This section describes the transfer parameters.

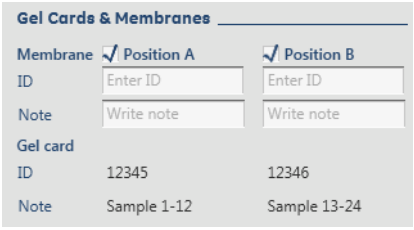
Enter transfer sandwich assembly information

Gel cards and membranes description

- In the ***TRANSFER*** workflow step in the software, in the ***Gel Cards & Membranes*** area, both ***Membrane Position A*** and ***Position B*** boxes are checked if two gel cards were run in the electrophoresis.

  Note: *If you only want to continue with one of the gel cards from the electrophoresis in the transfer, uncheck the appropriate box (**Membrane Position A** or **Position B**) in the **Gel Cards & Membranes** area.*

- If a ***Gel Card ID*** and a ***Gel Card Note*** have been entered for the gel card in the ***ELECTROPHORESIS & GEL SCANNING*** workflow step, these are displayed in their respective fields.



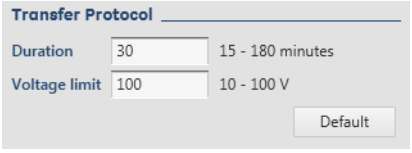
Enter membrane information

Perform the following steps for each position to be used in the transfer:

Step	Action
1	To enter the ID of a membrane, look at an Amersham WB PVDF card package. The ID information is found on the label of the PVDF card single package. Place the cursor in the ***ID*** field for the appropriate position in the software and: • scan the data matrix code on the PVDF card single package using the data matrix tag reader **or** • type in the ID manually in the software See also *Prepare the PVDF card, on page 188* for more information about how to prepare the PVDF card. ***Note:*** *When preparing the transfer sandwich(es), make sure that the gel card and the membrane for position A in the instrument/software are used together and the gel card and membrane for position B in the instrument/software are used together when assembling the transfer sandwiches.* *See Section 4.6.2 Prepare the transfer sandwich, on page 186 for information about how to assemble the transfer sandwich.* ***Note:*** *If a data matrix tag reader is a used, Code number and Lot number of the membrane will be logged in the* ***Experiment Information*** *dialog.*
2	To enter a note about the membrane, place the cursor in the ***Note*** field and type in a note.

View/edit the transfer protocol

Note: *The default settings for the transfer protocol do not normally need to be edited.*



If you need to edit any settings in the ***Transfer Protocol*** area, the table below gives a description of the settings. To revert to default values click the ***Default*** button.

Command	Description
Duration	Shows the duration time for the transfer run. ***Tip:*** *It is possible to increase the duration time if your sample contains larger proteins and decrease the duration time if you have smaller proteins.*
Voltage limit [1]	Shows the maximum voltage to be used during the run.
Default	Click to revert to default transfer protocol. A dialog asking for your confirmation will be displayed.

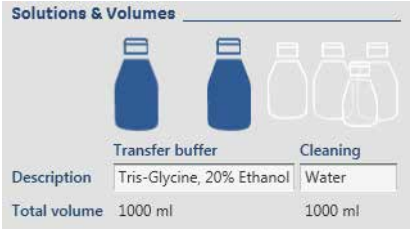
1 Maximum power is 40 W. Maximum current is 400 mA.

Enter information about the transfer solutions

The ***Description*** field shows the recommended ***Transfer buffer*** and ***Cleaning*** (ultra pure water) solutions to be used for a run.

Edit the descriptions if you will use other solutions in the run.

See *Transfer solution recipes, on page 103* for information about recipes.



4.3.5 Set up the probing protocol

Introduction

This section describes how to view/edit the probing protocol. No specific parameters need to be set for drying.

View/edit membrane information

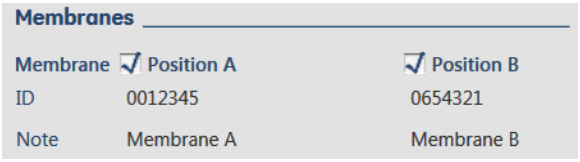
In the ***Membranes*** area:

- Both ***Membrane Position A*** and ***Position B*** boxes are checked if two PVDF cards were run in the transfer.

  If you only want to continue with one of the membranes from the transfer run, deselect the appropriate check box (***Membrane Position A*** or ***Position B***).

  Results:

  - The information for an unused position in the ***Antibodies*** area will be dimmed.
  - The software will automatically calculate the volume/solution to be used in the run according to that one membrane will be used.
- If an ***ID*** or ***Note*** was entered in the ***TRANSFER*** workflow step, these are displayed in their respective fields.



View antibodies information

In the ***Antibodies*** area (for the included positions):

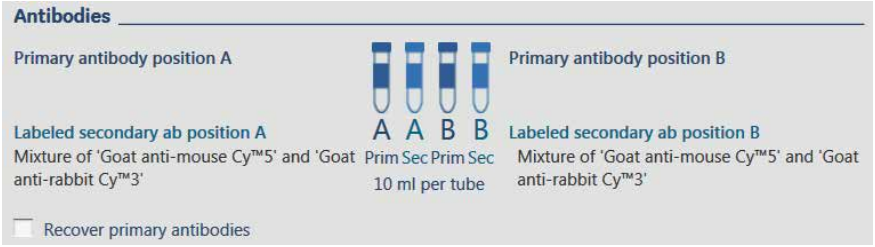
- The descriptions of the ***Primary antibody position A/position B*** are displayed (if they have been entered in the ***EXPERIMENT & SAMPLES*** workflow step).
- Under the ***Labeled secondary ab position A*** (and/or B):

  The secondary antibody to be used is displayed (e.g., Cy5 labeled anti-mouse antibody).

If two primary antibodies are used for one position, both antibodies are displayed. Then, the antibodies must be mixed in the antibody solution tube to be used in the position **A Primary** (or **B Primary**) in the antibody compartment. The same applies if two secondary antibodies are used.

Note: *The type of secondary antibody to be used need to be entered in the gel card tab before probing can be started. If there are two primary antibodies used, the selection in the **EXPERIMENT & SAMPLES Gel card** tab **A** and **B** will determine which antibody is displayed as target and which is displayed as control in further analysis. The first selected primary antibody will be shown in the membrane target image and the second selected primary antibody will be shown in the membrane control image.*

Note: *The antibody solution tube positions are marked with **A Primary/Secondary** and **B Primary/Secondary** on the inside of the lid. Make sure that tubes in positions A/B in the software/antibody compartment match the PVDF card positions A/B in the software/probing compartment.*



Recovery of primary antibodies

If you want to recover your primary antibodies, check the ***Recover primary antibodies*** box in the ***Antibodies*** area.

Recovered primary antibodies are automatically re-collected in their respective antibody solution tubes in the antibody compartment.

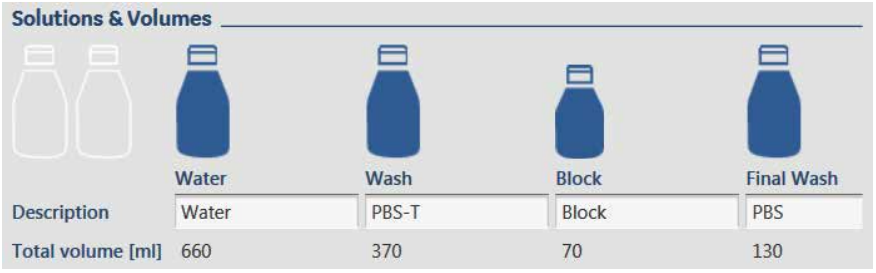
Note: *Not all of the primary antibody solution volume can be recovered and the recovered solution is diluted about 30%.*

Enter information about the probing solutions

In the ***Solutions & Volumes*** area, default descriptions of the ***Water***, ***Wash***, ***Block*** and ***Final Wash*** solutions are displayed.

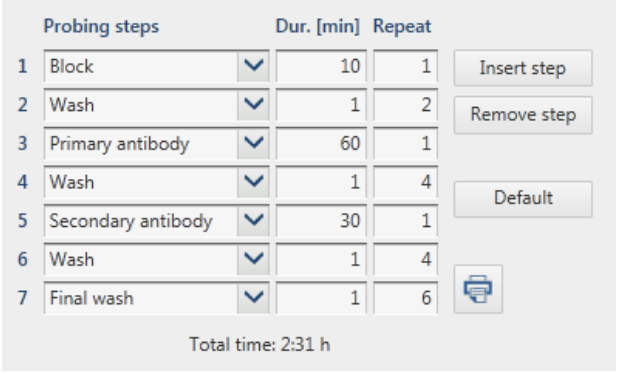
If you are using other solutions, edit the ***Description*** field(s) for the appropriate solution(s).

Note: *Make sure the **Water** bottle is filled with ultrapure water. The solution is used for rinsing of flow path between the probing steps. **Wash** solution is used for rinsing between primary antibody to position **A** and **B**.*



Probing protocol

In the ***Probing*** area, the probing sequence table is displayed showing the default steps for probing.



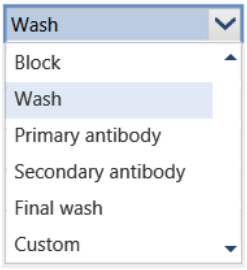
For each step, the following is shown:

- the solution to be used
- the duration time for the step
- how many times the step will be performed

If you want to edit an existing step or the sequence of steps, continue with the instructions below.

Edit a step in the probing protocol

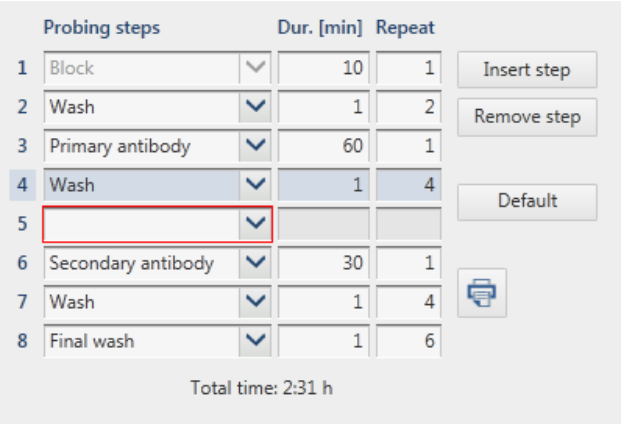
To edit information for an existing step:

Step	Action
1	To change the solution to be used, click the drop-down arrow and select the appropriate solution inlet.  ***Tip:*** *The **Custom** inlet can be used for any additional probing solution. It is not used in the default probing protocol. When selected, 16 ml of the custom probing solution will be pumped into the probing chamber. The custom inlet tubing is marked with **P Custom**.*
2	To change the duration time for a step, click in the appropriate ***Dur. (min)*** field and enter a new time.
3	To edit the number of times for a step to be performed, click in the appropriate ***Repeat*** field and edit the number.

Insert a new step in the probing protocol

To insert a step in the probing sequence:

Step	Action
1	Select the row in the table, below which you want to add a step.
2	Click ***Insert step***. *Result:* A new row is added.
3	Select the solution to be used in the ***Probing steps*** column drop-down list and enter duration time and the number of times the step should be performed. ***Note:*** *The duration and repeats for the default protocol are automatically presented on choosing the probing step.*



Remove a step from the probing protocol

To remove a step from the probing sequence:

- Select the step to be removed in the probing sequence table.
- Click ***Remove step***.

Print the probing information

To print the entered probing information, click the ***Print*** icon below the probing sequence table, or select ***File:Print*** in the menu bar, or press **Ctrl + P**. Select printer in the ***Print*** dialog and print out the information.

The following information will be printed:

- experiment name
- PVDF card information
- the antibodies to be used for probing and whether recovery of primary antibodies will be performed
- the probing sequence
- the solutions and volumes to be used in the probing run

4.3.6 View membrane scanning setup

Introduction

This section describes the membrane scanning parameters.

View membrane information

If an ***ID*** or ***Note*** was entered in the ***Membranes*** area in the ***TRANSFER*** workflow step, these are displayed in their respective fields.

View/edit scanning sensitivity parameters

In the ***Scanning Sensitivity*** area, the radio button ***Automatic sensitivity*** is selected by default.

Note: *This setting do not normally need to be edited.*

If you need to edit any settings in the ***Scanning Sensitivity*** area, the table below gives a detailed description of the settings.

Command	Description
Automatic sensitivity	Select this radio button to use the automatic sensitivity function at membrane scanning. The scanner and software automatically optimize the scanning sensitivity level for the membrane images. The sensitivity level is set so that the strongest protein signals in the membrane image do not become saturated (the outer most lanes are not used when sensitivity is optimized). This radio button is selected by default.
Manual sensitivity	Select this button to set/change the sensitivity level of the membrane scanning manually. For example: • if you get saturated signals for your protein of interest, try a lower sensitivity level and re-scan the membranes • if you get weak signals for your protein of interest, try a higher sensitivity level and re-scan the membranes Up to four gel scans per position can be saved within an experiment. Change the sensitivity level by dragging the ***Cy5 sensitivity level*** and/or ***Cy3 sensitivity level*** slider.
Default	Click to revert to default setting ***Automatic sensitivity***. A dialog asking for your confirmation will be displayed.

4.3.7 View or enter experiment information

Introduction

Experiment information can be entered/edited at any time during an experiment.

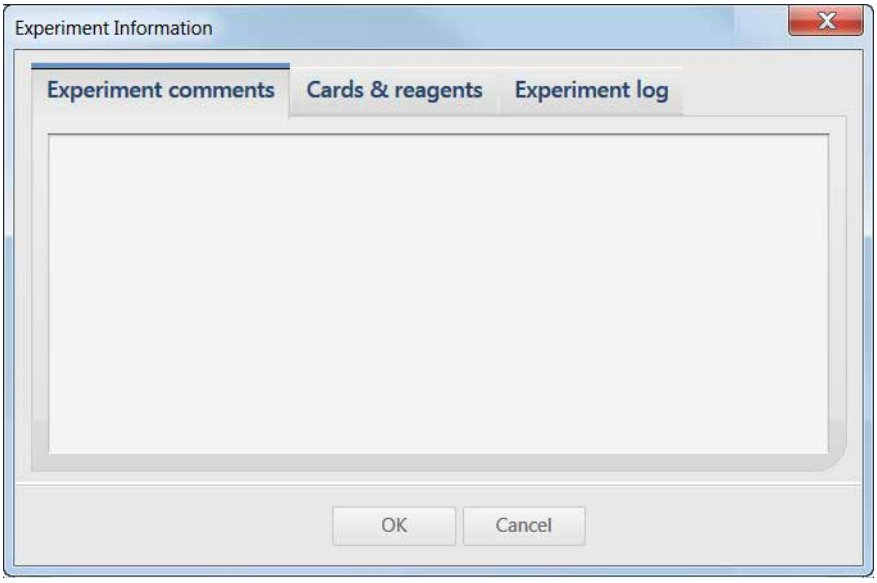
This section describes how to:

- enter general comments for an experiment
- view information about gel cards and PVDF cards
- add/remove custom consumables
- view the experiment log

Open the Experiment information dialog

In the software workflow area to the left, click the experiment information icon.

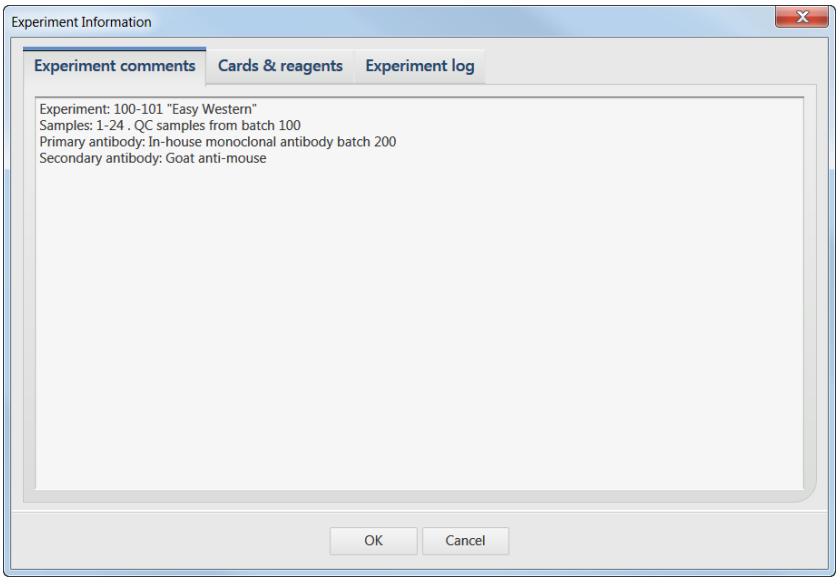
Result: The ***Experiment information*** dialog opens displaying the ***Experiment comments*** tab.



View/enter general experiment comments

- On the ***Experiment comments*** tab:

  Enter any general comments about the experiment. Previously entered comments can be viewed.



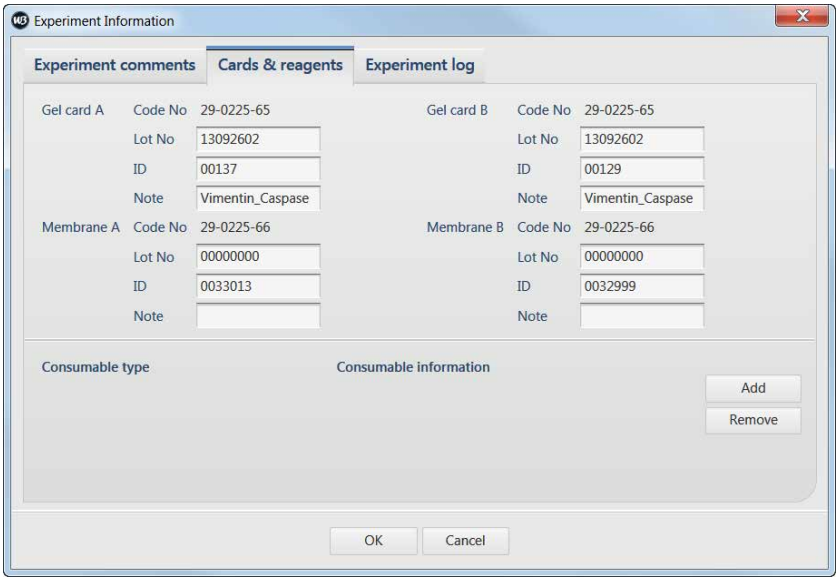
- Click ***OK*** to save any changes and to close the dialog.

View/enter information about gel cards and PVDF cards

In the ***Cards & reagents*** tab previously entered information about the gel cards and membranes in the ***ELECTROPHORESIS & GEL IMAGING*** and ***TRANSFER*** workflow steps is displayed. If the data matrix code on the gel card/PVDF card was scanned using the data matrix tag reader, the ***Code No*** (code number), ***Lot No*** and ***ID*** are automatically filled in on this tab.

- On the ***Cards & reagents*** tab:

  Enter or edit ***Lot No*** and ***ID*** information for the gel card/PVDF card. The ***Code No*** cannot be edited or entered by the user.



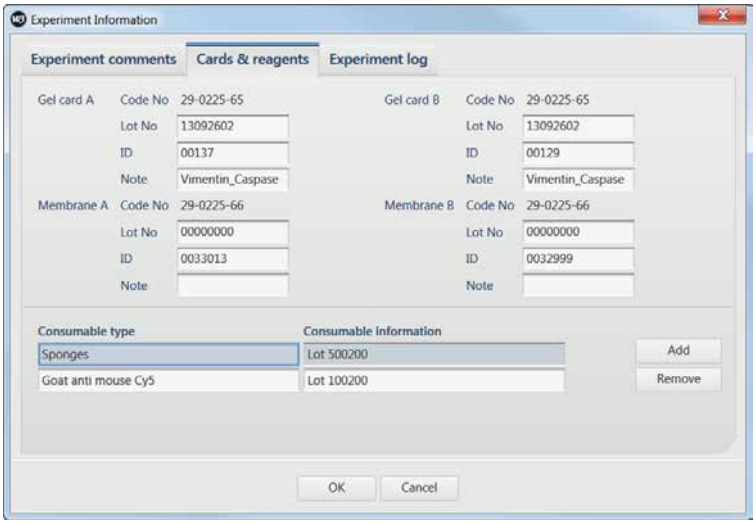
- Click ***OK*** to save any changes and to close the dialog.

Add a consumable to the Cards & reagents tab

To add a new consumable to the ***Cards & reagents*** tab, for example, a buffer that is used in the experiment:

Step	Action
1	Click the ***Add*** button. *Result:* A new row for entering consumable information is displayed.
2	Enter a description of the consumable in the ***Consumable type*** field.

Step	Action
3	Enter information about the consumable (e.g., lot number) in the ***Consumable information*** field.
4	Click ***OK*** to save any changes and to close the dialog.



Remove a consumable from the Cards & reagents tab

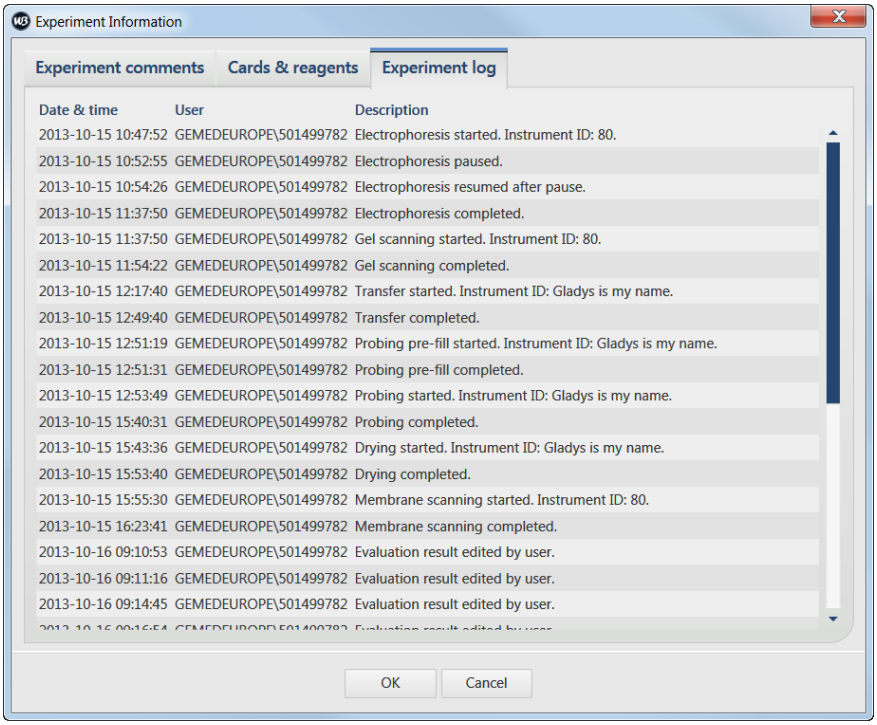
To remove a manually entered consumable:

- select the row with the consumable to be removed,
- click the ***Remove*** button,
- click ***OK*** to save any changes and to close the dialog.

View the experiment log

On the ***Experiment log*** tab, information about the events in the experiment is automatically logged.

For each event, the date and time for the event, the user logged in and a description of the event are displayed.


Experiment Information
Experiment comments
Cards & reagents
Experiment log
Date & time
User
Description
2013-10-15 10:47:52 GEMEDEUROPE\501499782 Electrophoresis started. Instrument ID: 80.
2013-10-15 10:52:55 GEMEDEUROPE\501499782 Electrophoresis paused.
2013-10-15 10:54:26 GEMEDEUROPE\501499782 Electrophoresis resumed after pause.
2013-10-15 11:37:50 GEMEDEUROPE\501499782 Electrophoresis completed.
2013-10-15 11:37:50 GEMEDEUROPE\501499782 Gel scanning started. Instrument ID: 80.
2013-10-15 11:54:22 GEMEDEUROPE\501499782 Gel scanning completed.
2013-10-15 12:17:40 GEMEDEUROPE\501499782 Transfer started. Instrument ID: Gladys is my name.
2013-10-15 12:49:40 GEMEDEUROPE\501499782 Transfer completed.
2013-10-15 12:51:19 GEMEDEUROPE\501499782 Probing pre-fill started. Instrument ID: Gladys is my name.
2013-10-15 12:51:31 GEMEDEUROPE\501499782 Probing pre-fill completed.
2013-10-15 12:53:49 GEMEDEUROPE\501499782 Probing started. Instrument ID: Gladys is my name.
2013-10-15 15:40:31 GEMEDEUROPE\501499782 Probing completed.
2013-10-15 15:43:36 GEMEDEUROPE\501499782 Drying started. Instrument ID: Gladys is my name.
2013-10-15 15:53:40 GEMEDEUROPE\501499782 Drying completed.
2013-10-15 15:55:30 GEMEDEUROPE\501499782 Membrane scanning started. Instrument ID: 80.
2013-10-15 16:23:41 GEMEDEUROPE\501499782 Membrane scanning completed.
2013-10-16 09:10:53 GEMEDEUROPE\501499782 Evaluation result edited by user.
2013-10-16 09:11:16 GEMEDEUROPE\501499782 Evaluation result edited by user.
2013-10-16 09:14:45 GEMEDEUROPE\501499782 Evaluation result edited by user.
OK
Cancel

4.3.8 Save experiment

Introduction

An experiment can be saved as an experiment file (*.AWBexp). This section describes when and how to save an experiment.

When to save an experiment?

You must save the experiment before a run is started. If the experiment has not been saved when starting a run, the ***Save as*** dialog appears where you can save the experiment.

When the experiment file has been created, the experiment will automatically be saved before and after every run.

Save an experiment

To save the experiment the first time:

- Select ***File:Save*** in the menu bar.

  Result: The ***Save as*** dialog opens.

- Type in a name for the experiment and click ***Save***.

To save an existing experiment, select ***File:Save*** in the menu bar or press **Ctrl+S**.

Note: *It is recommended to save the experiment to a local hard drive during a run to avoid network connectivity problems.*

Save an experiment with a new name

To save an existing experiment with a new name:

- Select ***File:Save as*** in the menu bar.

  Result: The ***Save as*** dialog opens.

- Type in a name for the experiment and click ***Save***.

4.4 Prepare samples

Introduction

Samples can be either unlabeled or pre-labeled depending on the application.

- The Easy SDS-PAGE and Western with total protein normalization experiment types require pre-labeling of samples.
- In the Easy Western and Western with endogenous protein normalization experiment types samples are not pre-labeled.

This section describes how to perform pre-labeling of samples and how to prepare unlabeled samples.

In this section

This section contains the following subsections:

4.4.1 Perform pre-labeling of samples

Introduction

Pre-labeling of samples is required for detection when running electrophoresis experiments. It is also required in Western experiments when detecting the total protein on a membrane.

This section describes:

- pre-requisites for pre-labeling
- materials required for pre-labeling
- preparations before starting pre-labeling
- labeling of samples (pre-labeling protocol)

For more information about pre-labeling, see *Section 3.2 Pre-labeling of proteins, on page 83*.

Precautions

WARNING

When working with pre-labeling of protein samples:

- Always wear protective clothing, gloves and protective glasses.
- Read the Safety Data Sheet (SDS/MSDS) before performing pre-labeling of samples.

CAUTION

When pre-labeling of proteins in samples has been performed a strong odor may arise from trace amounts of dimethyl sulfide (DMS) and dimethyl sulfoxide (DMSO). Local exhaust ventilation may be required. Follow local regulations and instructions for safe operation.

Note: *To obtain comparable labeling efficiencies, important parameters such as pH, reaction volume, temperature and buffer salts should be invariant.*
Labeling efficiencies will also vary from protein to protein.

Pre-requisites for pre-labeling and protocol selection

See *Section 3.2.2 Pre-labeling pre-requisites and protocols, on page 86* for information about pre-requisites for pre-labeling and how to select protocol.

Required solutions and materials

The following solutions and materials are required for the pre-labeling of proteins:

- Pre-labeling consumables: Cy5 dye reagent, Labeling buffer, Loading buffer
- 1 M DTT (dithiothreitol) stock solution (DTT and ultra pure water) if reducing SDS-PAGE will be performed
- Ultra pure water for diluting the Cy5 dye reagent
- Original lysis buffer for adjusting reaction volume (Western experiments only)
- 0.5 ml microfuge tubes
- Heating block
- Vortex
- Centrifuge

Preparations before starting pre-labeling

Step	Action
1	• Take out one of each of the following vials (enough for running two gel cards) from the freezer: - 1 vial Amersham WB Cy5 - 1 vial Amersham WB labeling buffer (not needed if labeling samples using the Western pre-labeling protocol) - 1 vial Amersham WB loading buffer • Thaw the pre-labeling components completely. • Equilibrate the Cy5 vial to room temperature before opening to avoid moisture condensation.
2	Briefly spin down the Cy5 dye reagent liquid using a centrifuge.
3	Set the temperature of the heating block to 95°C.
4	For reducing SDS-PAGE, add reducing agent to the Loading buffer: • Add 29 µl 1 M DTT stock solution to 0.7 ml (one vial) Loading buffer, and vortex to mix.

Note: *Prepare the Amersham WB molecular weight markers at the same time. See Section 4.4.3 Prepare Amersham WBmolecular weight markers, on page 162.*

Pre-labeling of quantity calibrants

If purified protein samples are used as quantity calibrants, label each calibrant individually (i.e., one labeling reaction for each calibrant) for best possible quantity calibration.

Also, samples and quantity calibrants should be pre-labeled under the same conditions.

Note: *The slope and linearity of the calibration curve depends both on the protein and the sample concentration range. For quantity calibration, use the same calibrant protein as the sample protein of interest.*

Perform sample pre-labeling (SDS-PAGE pre-labeling protocol)

Note: *To adjust the amount of sample loaded per well (maximum 0.5 µg/protein band for optimal resolution), dilute the sample prior to, or after, pre-labeling. The Amersham WB labeling buffer is used to dilute samples prior to pre-labeling. For dilution after labeling, use the Amersham WB loading buffer (with or without DTT) diluted with an equal volume of ultra pure water. For pre-labeling of samples with a small number of proteins, a suitable starting concentration is 1 mg/ml. The protocol below can be scaled up.*

Step	Action
1	If the protein sample contains interfering substances, perform a buffer exchange using Amersham WB MiniTrap kit.
2	Perform labeling reaction in a 0.5 ml microfuge tube: • **For protein samples:** First add 17 µl of labeling buffer and then 2 µl of protein sample (1 ng/µl - 20 µg/µl) and mix. [1] • **For proteins samples after buffer exchange:** Add 2-19 µl of protein sample (1 ng/µl - 20 µg/µl in Labeling buffer) to the tube. Fill up to a volume of 19 µl using Labeling buffer.
3	Heat the samples at 95ºC for 1 minute using a heating block. [2]
4	• Remove the microfuge tubes from the heating block. • Let the samples cool down for 5 minutes at room temperature.
5	• Add 1 µl of Cy5 dye reagent. • Mix thoroughly by quickly vortexing.
6	Incubate at room temperature for 30 minutes. ***Note:*** *It is important to make sure that the labeling volume and time are equal for all samples.*
7	Add 20 µl of Loading buffer.
8	Heat the samples at 95ºC for 3 minutes.

Step	Action
9	Spin down the samples. Total sample volume is 40 µl per reaction (recommended loading volume is 20 µl). Proceed with the instructions in *Section 4.5 Perform electrophoresis and gel scanning, on page 163*. If the electrophoresis analysis will be performed at a later stage, store the pre-labeled samples at -20°C.

1 A sample volume of 2 µl is sufficient for most protein samples with concentrations ranging from 1 ng/µl to 20 µg/µl. The 10-fold dilution in Labeling buffer ensures optimal labeling conditions.

2 It is possible to skip the heating step for temperature sensitive samples

Perform sample pre-labeling (Western pre-labeling protocol)

Tip: *The protocol below can be scaled up.*

Step	Action
1	Perform the labeling reaction in a 0.5 ml microfuge tube: • Add 2-19 µl cell lysate or tissue extract sample (maximum 40 µg total protein), and fill up to a volume of 19 µl using original sample lysis buffer. • Add 1 µl of Cy5 dye reagent diluted 1:10 in ultra pure water. ***Note:*** *The diluted dye must be freshly prepared and used within 30 minutes. It can not be frozen and re-used.* • Briefly vortex to mix thoroughly.
2	Incubate at room temperature for 30 minutes. ***Note:*** *Make sure that the labeling time and volume is equal for all samples.* *For temperature sensitive samples, incubate on ice for 30 minutes.*
3	Add 20 µl of Loading buffer.
4	Heat the samples at 95ºC for 3 minutes.
5	Spin down the samples. Total sample volume is 40 µl per reaction (recommended loading volume is 20 µl). Proceed with the instructions in *Section 4.5 Perform electrophoresis and gel scanning, on page 163*. If the electrophoresis analysis will be performed at a later stage, store the pre-labeled samples at -20°C.

Perform sample pre-labeling (Quick SDS-PAGE pre-labeling protocol)

Note: *To adjust the amount of sample loaded per well (maximum 0.5 µg/protein band for optimal resolution), dilute the sample prior to, or after, pre-labeling. The Amersham WB labeling buffer is used to dilute samples prior to pre-labeling. For dilution after labeling, use the Amersham WB loading buffer (with or without DTT) diluted with an equal volume of ultra pure water. The reaction below can be scaled up.*

Step	Action
1	If the protein sample contains interfering substances, perform a buffer exchange using Amersham WB MiniTrap kit.
2	Perform labeling reaction in a 0.5 ml microfuge tube: • **For protein samples:** First add 17 µl of labeling buffer and then 2 µl of protein sample (1 ng/µl - 20µg/µl) and mix. [1] • **For proteins samples after buffer exchange:** Add 2-19 µl of protein sample (1 ng/µl - 20µg/µl in Labeling buffer) to the tube. Fill up to a volume of 19 µl using Labeling buffer.
3	• Add 1 µl of Cy5 dye reagent. • Mix thoroughly by a quick vortex.
4	Incubate at 95ºC for 3-5 minutes.
5	Add 20 µl of Loading buffer.
6	Heat the samples at 95ºC for 3 minutes.
7	Spin down the samples. Total sample volume is 40 µl per reaction (recommended loading volume is 20 µl). Proceed with the instructions in *Section 4.5 Perform electrophoresis and gel scanning, on page 163*.

1 A sample volume of 2 µl is sufficient for most protein samples with concentrations ranging from 1 ng/µl to 20 µg/µl. The 10-fold dilution in labeling buffer ensures optimal labeling conditions.

4.4.2 Prepare unlabeled samples

Introduction

This section describes how to prepare unlabeled samples and sample loading capacity.

Prepare samples

Step	Action
1	Set the temperature of the heating block to 95°C.
2	For reducing SDS-PAGE, add reducing agent to the Loading buffer: • Add 29 µl 1 M DTT stock solution to 0.7 ml (one vial) Loading buffer and vortex (final concentration 40 mM).
3	Mix equal volume of Loading buffer and sample.
4	Heat the samples at 95°C for 3 minutes.

Sample loading capacity

The table below describes the loading capacities:

Loading volume/well	Protein amount
15-30 µl [1] 20 µl, recommended	Maximum 20 µg/well <0.5 µg/protein band

1 Difference between wells should be <10 µl. Adjust with Amersham WB loading buffer (diluted with an equal volume of ultra pure water).

4.4.3 Prepare Amersham WBmolecular weight markers

Introduction

Take out one vial of Amersham WB molecular weight markers (molecular weight markers) (enough for running two gel cards) from the freezer and thaw completely.

Western experiments

In Western experiments, Amersham WB molecular weight markers should be diluted 1:20 in Loading buffer before loading on the gel card. Before use, the supplied Loading buffer must be diluted with ultra pure water and contain added DTT.

Step	Action
1	Add 29 µl of 1 M DTT stock solution to 0.7 ml (one vial) Loading buffer. Vortex to mix.
2	Dilute the DTT-containing Loading buffer with an equal volume of ultra pure water. Vortex to mix.
3	Dilute the molecular weight markers 1:20 with the prepared Loading buffer.

Note: *The molecular weight markers can be diluted further in prepared Loading buffer to match samples with weak signals. However, it is then necessary to select the option* ***Stop electrophoresis on time****.*

Electrophoresis experiments

In Electrophoresis experiments, there is usually no need to dilute the Amersham WB molecular weight markers. If needed, dilute the molecular weight markers with Loading buffer containing DTT (diluted with an equal volume of ultra pure water).

Replicates

It is recommended to include two marker lanes in the run to obtain best possible results in the automatic molecular weight calibration of sample proteins in the evaluation step.

4.5 Perform electrophoresis and gel scanning

Introduction

This section describes how to perform protein electrophoresis including:

- preparations before starting electrophoresis
- start and monitor electrophoresis and gel card scanning
- procedures after electrophoresis and gel card scanning

In this section

This section contains the following subsections:

4.5.1 Preparations before starting electrophoresis

Introduction

This section describes how to load the buffer strips, gel card and samples for the electrophoresis run.

Preparations before starting electrophoresis

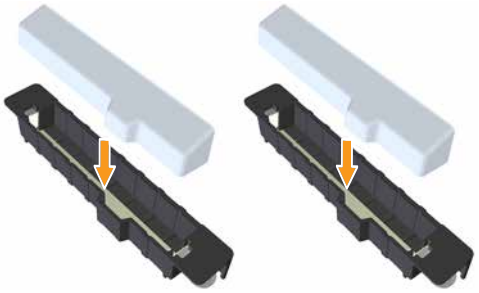
WARNING

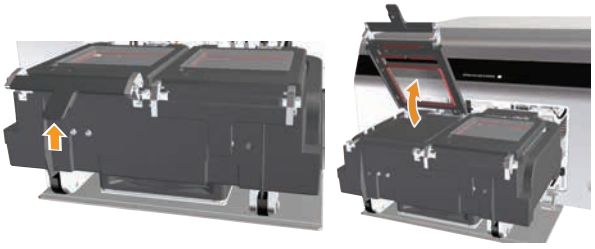
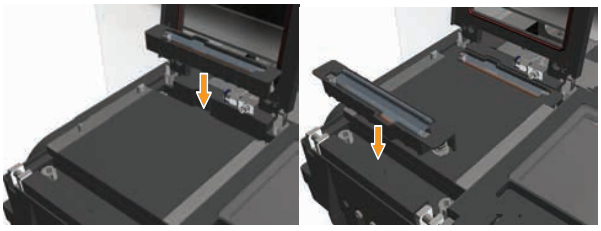
Exploding glass. Do not run the system if the glass in the sealing lid in the Elpho & scan unit is scratched or broken. Switch off the instrument, disconnect the power cord and contact an authorized service engineer.

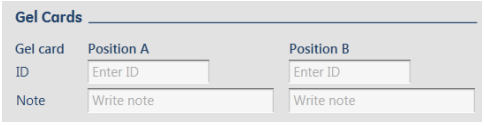
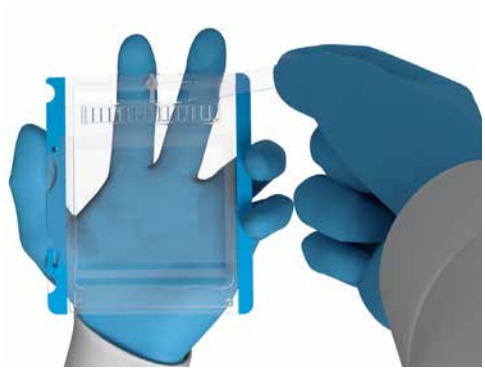
CAUTION

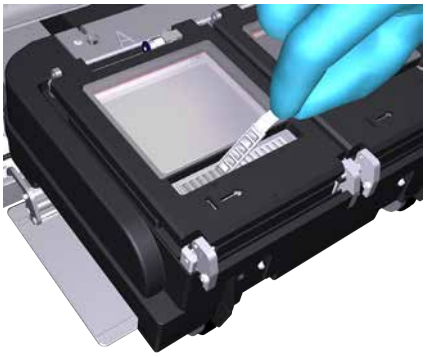
Moving parts. Be careful when opening/closing the loader to avoid fingers or clothing becoming trapped when the loader is moving.

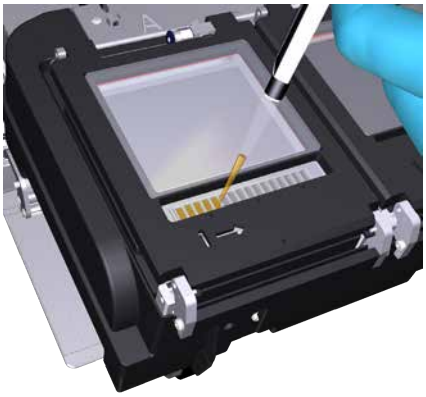
Never place any bottles or vials in front of the Elpho & scan unit. They may fall when the loader is opened.

Step	Illustration of step	Operator actions
1		Place the buffer strips in the buffer strip holders by pushing out two buffer strips from their package directly into the buffer strip holders. ***Note:*** *To avoid contamination of the buffer strips, do not touch the buffer strips, or make sure to use clean gloves.*

Step	Illustration of step	Operator actions
2		Press the eject button on the Elpho & scan unit. *Result:* The loader is ejected.
3		Open the sealing lid using the latch.
4		Place the buffer strip holders in the card plate cavities in the loader.

Step	Illustration of step	Operator actions
5	 	• Take out a gel card from its package (with the front side facing up). • *Optional:* Place the cursor in the ***ID*** field in the software at the ***ELECTROPHORESIS & GEL SCANNING*** workflow step of the software and scan the gel card matrix tag (indicated with an orange circle) using the matrix tag reader. See *Enter gel card information , on page 131* for more information.
6	 	• Turn the gel card so that the reverse side is facing up (blue gel handles without labels are facing up). • Grip the end of the protective film at the bottom of the gel card and carefully remove it. • Grip the end of the protective film at the top of the gel card and carefully remove it. By removing the protective films, contact between the gel and buffer is established when placing the gel card on the card plate. Be careful not to touch the exposed gel surfaces.

Step	Illustration of step	Operator actions
7		• Turn the gel so the front side is facing up (i.e., sample well cover is facing up). ***Note:*** *Make sure the card plate is completely dry.* • Place the gel card on the card plate by aligning the holes on the gel card frame with the guiding pins marked with orange circles. ***Note:*** *Be careful not to scratch the card plate with any sharp objects.*
8		• Close the sealing lid. • Grip the end of the sample well cover and carefully remove it. ***Tip:*** *Change grip while removing the sample well cover to be able to hold as near the wells as possible.* ***Note:*** *If the sample well cover breaks: Let the gel card remain in the loader with the sealing lid closed. Use for example tweezers to remove the rest of the well cover.*

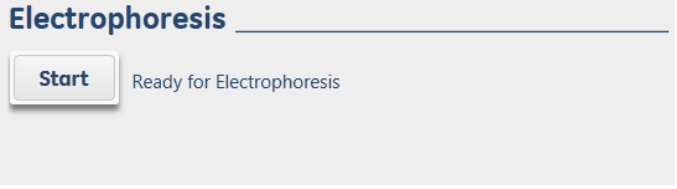
Step	Illustration of step	Operator actions
9		Load the samples, the Loading buffer, and/or the molecular weight markers into the wells (typically 20 µl/well) as set up in the ***EXPERIMENT & SAMPLES*** screen in the software. ***Note:*** *Never open the lid after the samples have been loaded.* ***Note:*** *All wells must be loaded with a sample, Loading buffer (diluted with an equal volume of ultra pure water) or molecular weight markers. It is recommended to have Loading buffer in wells 1 and 16, and molecular weight markers in wells 2 and 15.*
10		When all samples have been loaded, press the eject button. *Result:* The loader is inserted into the Elpho & scan unit.

4.5.2 Run electrophoresis

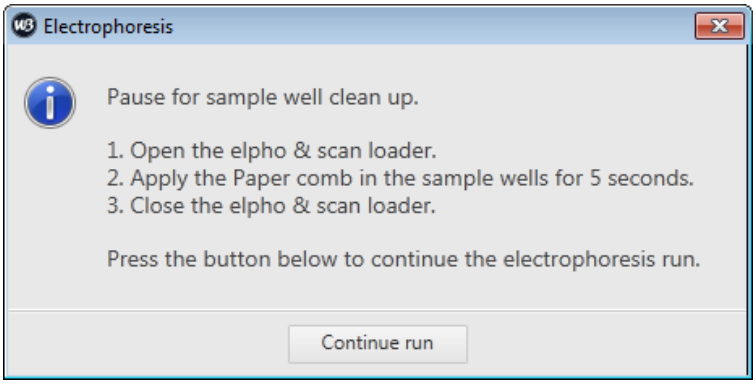
Introduction

This section describes how to start and monitor the electrophoresis. How to view and re-scan gel images is also described.

Run electrophoresis and gel scanning

Step	Action
1	Make sure that: • buffer strip holders containing the buffer strips have been placed into the Elpho & scan unit • gel card(s) have been placed on the card plate • samples have been loaded in the gel card(s) wells • the loader has been closed
2	Click ***Start*** in the ***Electrophoresis*** area.  *Result:* The run is started. The indicator lamp on the Elpho & scan unit changes to a blue triangle. ***Note:*** *If you have not saved the experiment earlier, the **Save as** dialog opens. The experiment must be saved before the run can be started.*

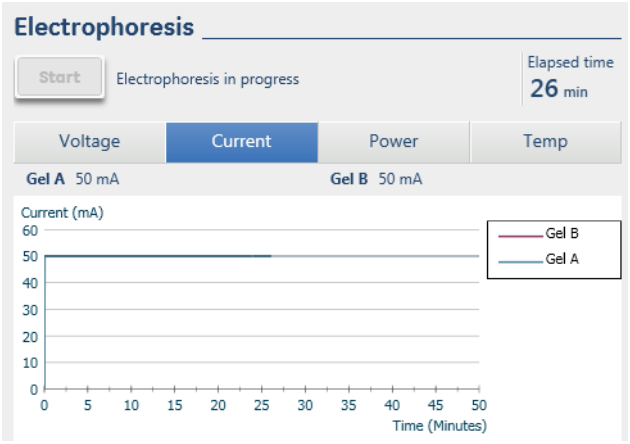
Step	Action
3	If sample well cleanup has been selected, the electrophoresis will stop after the calculated time and a dialog is displayed.



- Eject the Elpho & scan loader.
- Apply an Amersham WB paper comb into the wells of the gel card for 5 seconds.

- Close the Elpho & scan loader.
- To continue the electrophoresis, click ***Continue run***.

Step	Action
4	The progress of the electrophoresis run is viewed in the ***Electrophoresis*** area and graphs.



- **Graphs**

  By default, the graph shows the current for the progressing run. Graphs for both gel card positions are displayed in the same view (color coded lines).

  Click a tab (***Voltage***, ***Current***, ***Power***, ***Temp***) to view the corresponding graph for that parameter and the progress of the run.

- **Pause at and remaining time**

  The time for the pause when sample well clean up will be performed (optional) is displayed.

- The ***Elapsed time*** is shown above the graph to the right if using front detection to stop the electrophoresis run.

  If a manually set time has been entered for stopping the electrophoresis run, ***Remaining time*** and when the run will be completed (***Ready at***) is displayed instead of ***Elapsed time***.

- **Run status**

  The status of the run is displayed to the right of the ***Start*** button. See *Section 2.9.5 Instrument status panel, on page 76* for information about the instrument status panel and status messages.

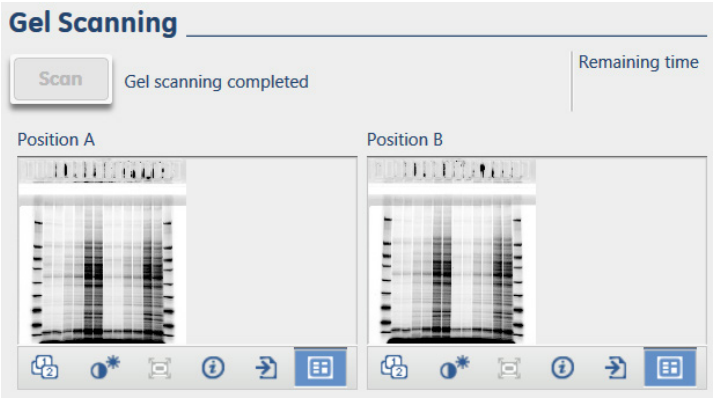
  During the run, the message ***Electrophoresis in progress*** is displayed.

  When the run has finished the status to the right of the ***Start*** button is changed to ***Electrophoresis completed***. The indicator lamp on the Elpho & scan unit changes to a white square.

Step	Action
	Tip: *The progress of the run can also be viewed in the instrument status panel at the bottom of the screen. See Section 2.9.5 Instrument status panel, on page 76 for more information.* *The name of the experiment, the elapsed time/remaining time and the run status are displayed.* ***Note:*** *To abort a run, select* ***Control:Abort run*** *from the menu bar. When an electrophoresis has been aborted the protocol settings can be changed and the electrophoresis restarted.*
5	To copy a graph (during or after a run), right-click on the graph and select ***Copy Graph***. *Result:* The graph is copied to the clipboard. It can be pasted into another application, for example Microsoft Excel or Microsoft Word®.

Step	Action
6	When the electrophoresis has been completed:

- Gel scanning is automatically started, if this was selected in the ***Gel Scanning Protocol*** area. The scanned gel card images are displayed in the ***Gel Scanning*** area.



or

- If automatic gel scanning was not included in the the ***Gel Scanning Protocol***, it is still possible to scan the gel cards by clicking ***Scan*** in the ***Gel Scanning*** area.

Note:

To abort an ongoing scanning, select ***Control:Abort run*** *and click* ***OK*** *in the displayed dialog. When scanning has been aborted the scanning protocol can be modified and the scan restarted. The aborted image is included in the image stack.*

See instructions below for more information about the scanned images and how to re-scan images.

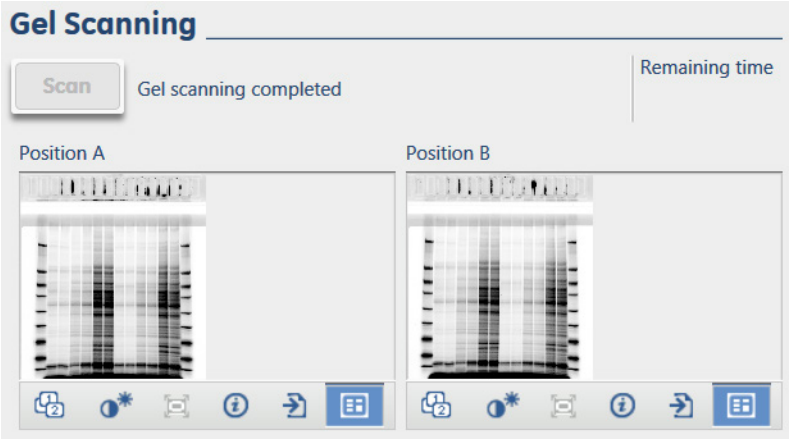
Note:

By default, automatic gel scanning is not included for ***Easy Western*** *and* ***Western with endogenous protein normalization*** *experiments because no pre-labeling of proteins is performed.*

For Western experiments where no gel scanning has been performed, see Section 4.5.3 Procedures after electrophoresis and gel card scanning, on page 182.

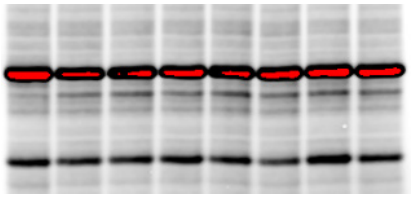
Overview – Gel image preview

When electrophoresis has been completed, gel scanning is automatically started if this was selected in the ***Gel Scanning Protocol*** area. The scanned gel card images are displayed in the ***Gel Scanning*** area.

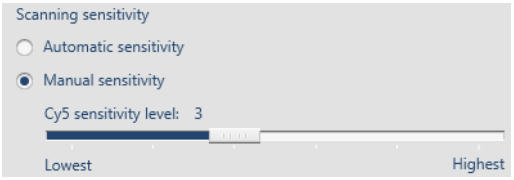
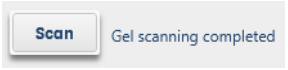


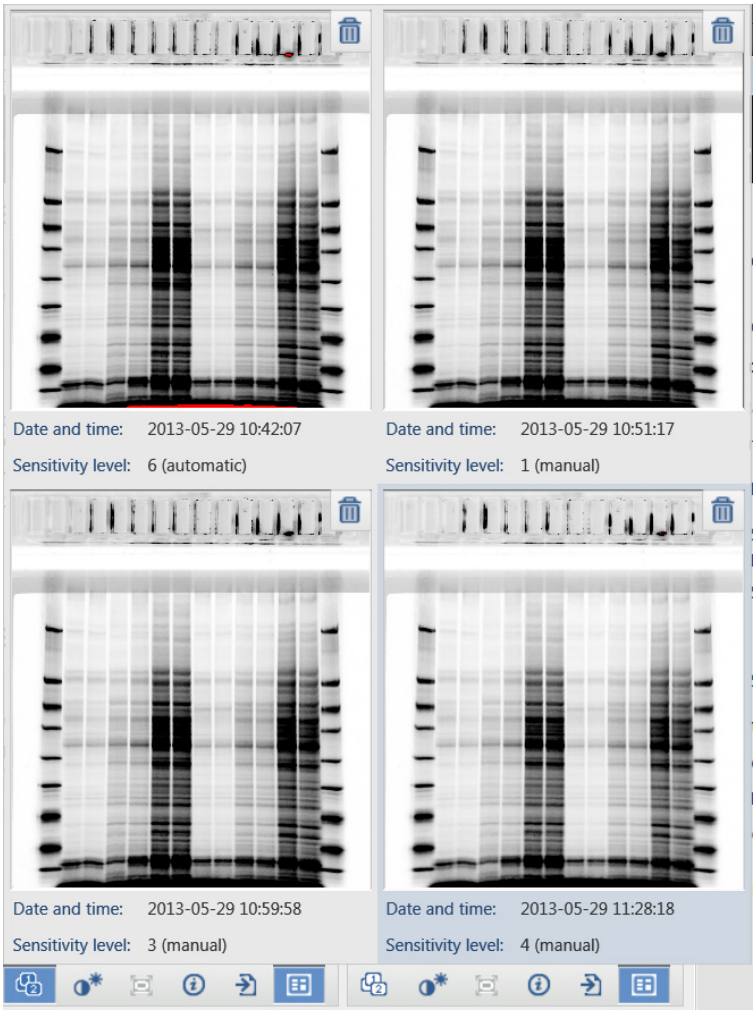
Information about how to zoom out of the image, adjust contrast and brightness, display image information, exporting an image and turning annotations on or off are described in *Tool descriptions – Gel Image Preview, on page 178*.

Assess the image quality

Step	Action
1	Check the scanned images. It is possible to zoom in on the image by holding down the left mouse button and dragging a rectangle over the gel area of interest. Saturated signals in the gel images are displayed in red (annotations must be turned on, see *Tool descriptions – Gel Image Preview, on page 178*).  • If you have saturated signals in the areas of interest of your gel images, consider re-scanning your gels using a lower sensitivity level. It is possible to calculate volumes, but the volumes of saturated areas should not be used for quantitative purposes. • If you have too weak signals in the areas of interest of your gel images, consider re-scanning your gel images using a higher sensitivity level.
2	If you need to re-scan your images, see *Re-scan of gel images, on page 176* for instructions.
3	If the images look fine, view the information in *Section 4.5.3 Procedures after electrophoresis and gel card scanning, on page 182* and then proceed with the: • ***EVALUATION*** workflow step (Electrophoresis experiments) • ***TRANSFER*** workflow step (Western experiments)

Re-scan of gel images

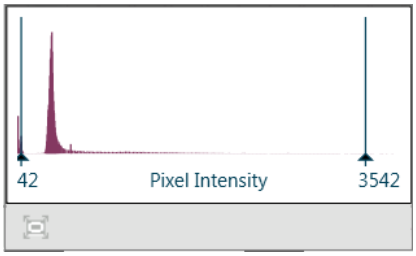
Step	Action
1	In the ***Gel Scanning Protocol*** area, select the ***Manual sensitivity*** radio button and drag the slider to the appropriate sensitivity level.  ***Tip:*** *The sensitivity level used at scanning for the currently displayed gel image can be viewed by clicking the Image information tool.* 
2	Click ***Scan*** to start scanning the gel cards with the new settings. 
3	When the scanning has been completed, the latest scanned images are displayed in the ***Gel Scanning*** area.

Step	Action
4	It is possible to compare the latest scanned gel image with previously scanned gel images by clicking the Image stack tool. *Result:* A window with all scanned images is displayed. For each image, the ***Date & Time*** and ***Sensitivity level*** and whether automatic or manual sensitivity was used at scanning are displayed. 
5	Choose the best gel image by clicking on the appropriate image. *Result:* The selected image is displayed in the ***Gel Scanning*** area. This image will be passed on to the ***EVALUATION*** workflow step.

Step	Action
6	• *Electrophoresis experiments:* Proceed with the ***EVALUATION*** workflow step to evaluate your results. • Western *experiments:* Proceed with the ***TRANSFER*** workflow step.

Tool descriptions – Gel Image Preview

Tool	Description
	Image stack tool. Click to display different scans of the same gel image. • To remove an image from the image stack, click the Delete symbol  at the top right corner of the image to be removed. • To select an image to display in the ***Gel Image Preview***, click the appropriate image. ***Note:*** *Four images per gel can be kept in the image stack at the same time.*

Tool	Description
	Contrast and brightness tool. Click to open the contrast and brightness control.  You can directly modify the brightness and contrast of the display by dragging the handles on the graph. • Moving the handles closer together increases the contrast (and vice versa) of the pixels within the range. See *Contrast and brightness, on page 180* for more information. • To reset any changes, click the  tool in the contrast and brightness control window. The change does not alter any raw data or calculations within the software. It is only a view setting, and optimum values are actually dependent on the current monitor.
	Zoom out tool. This tool is only enabled when an image has been zoomed. Click to fully zoom out of the image. ***Tip:*** *It is also possible to zoom out by double clicking in the image.* ***Tip:*** *Zoom in on an image area by holding down the left mouse button and dragging a rectangle over the gel area of interest. It is possible to zoom several times in an image.*
	Image information tool. Click to to display the ***Image information*** dialog. It shows data about the gel image currently displayed in the ***Gel Image Preview***.

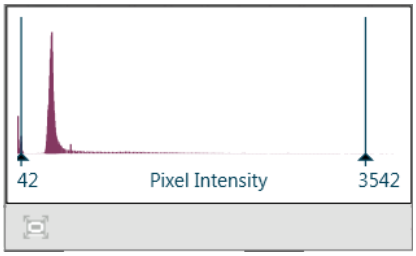
Tool	Description
	Export image tool. Select to export the image as: • Normal export (.tif), or • Compressed export (.jpg) In the ***Export Image*** dialog browse to the appropriate folder, type in a name for the image, and click ***Save***. ***Note:*** *When exporting to *.tif format, the contrast of the image may need to be adjusted before export in order to obtain the correct appearance in the software used (TIFF has over 65000 intensity levels and a normal screen 256).* ***Tip:*** *It is also possible to copy a gel image to the clipboard by right-clicking on the membrane image and selecting **Copy** (or press **Ctrl+C** on the keyboard). The image can then be pasted into another application.*
	Annotations tool. Click to turn on/off the annotations in the gel images. If the tool is blue it means that annotations are turned on. If the tool is white it means that annotations are turned off.

Contrast and brightness

The contrast and brightness control displays the frequency with which each pixel intensity occurs within the image. The peaks on the graph represent the pixel intensities that occur most frequently within the image.

The left and right handles on the graph show the range of pixel intensities in the image that will be mapped to a gray scale in the display image. Pixels with intensities below the left handle will be displayed as completely white in the image. Pixels with intensities above the right handle will be displayed as completely black in the image. Pixels between the handles will be displayed in various shades of gray.

The raw image can use up to 65536 intensity levels. The display normally displays 256 levels. The contrast and brightness translate between the wide range raw image and the display.



For information about how to change contrast and brightness, see *Tool descriptions – Gel Image Preview, on page 178*.

4.5.3 Procedures after electrophoresis and gel card scanning

Introduction

This section describes how to remove the gel card and clean the Elpho & scan unit after electrophoresis.

Remove gel cards

WARNING

After electrophoresis, or after transfer (when the transfer sandwich has been opened and the PVDF card has been placed in the probing compartment), dispose of the gel in a safe way. Read the gel card Safety Data Sheet (SDS/MSDS) for safety instructions regarding the gel and disposal of the gel.

Note: *If performing a Western experiment, the transfer run should be started within one hour after the completion of the electrophoresis.*

Step	Action
1	When the electrophoresis is ready and the white ready lamp is lit on the instrument panel, press the eject button on the Elpho & scan unit. *Result:* The loader is ejected.
2	Open the sealing lid using the latch.
3	Remove the gel card and place it upside down on the bench.
4	Proceed to *Clean the Elpho & scan unit, on page 183*. ***Note:*** *It is recommended to clean the Elpho & scan unit after each run. However if performing a Western experiment and you are short of time, proceed to transfer and clean the unit at a later stage of the experiment.*

Clean the Elpho & scan unit

Step	Action
1	Remove the buffer strip holders from the loader and discard the buffer strips.
2	Wipe off any liquid on the card plate using a lint-free cloth, if needed wetted with 50% ethanol.
3	Wipe off any liquid and dirt from the protective glass using a lint-free cloth, if needed wetted with 50% ethanol. ***Note:*** *It is important to keep the protective glass clean in order to obtain a good result when scanning gel cards and PVDF cards.*
4	Close the sealing lid and press the eject button on the Elpho & scan unit. *Result:* The loader is inserted.
5	Clean the buffer strip holders using running water to remove salts and buffer. Leave the buffer strip holders to air dry upside down.
6	Proceed to *Continue to transfer, on page 183*, if appropriate.

Continue to transfer

Do one of the following, if running a Western experiment:

- If transfer solutions have been prepared and connected to the Western unit, proceed with the instructions in *Section 4.6.2 Prepare the transfer sandwich, on page 186*.

  otherwise

- Proceed with the instructions in *Section 4.6.1 Prepare and connect transfer solutions, on page 185*.

4.6 Perform the Western Blot steps (Western experiments only)

Introduction

This section describes how to:

- prepare and connect solutions for transfer and probing
- assemble the transfer sandwich
- perform transfer, probing & drying and membrane scanning
- view the results

In this section

This section contains the following subsections:

4.6.1 Prepare and connect transfer solutions

Introduction

This section describes how to prepare and connect buffers and solutions for the transfer.

Transfer solutions can be prepared during the electrophoresis run and must be connected before starting the transfer.

Prepare transfer solutions

See *Transfer solution recipes, on page 103* for information about how to prepare solutions for the Western blot steps.

Connect tubing to the transfer buffer and ultra pure water on the bottle rack

CAUTION

To avoid the splashing of hazardous liquids, use heavy inlet filters connected to tubing in bottles.

See *Section 2.8 Other accessories, on page 61* for descriptions of inlet filters and tubing holders. See Section *Hardware installation* in *Amersham WB system Operating Instructions* for how to attach the accessories.

Before starting transfer, connect tubing to the transfer buffer and ultra pure water (used for cleaning the transfer tank) as follows:

Step	Action
1	From the left tubing tower, place the tubing marked **T Buffer** into a 1000 ml bottle with transfer buffer.
2	Place the the tubing marked **T Water** into a 1000 ml bottle with ultra pure water.

4.6.2 Prepare the transfer sandwich

Required materials

The following consumables and accessories are required when preparing one transfer sandwich:

- 1 transfer holder
- 1 gel card that has been run
- 1 PVDF card
- 2 sponges
- 2 transfer papers
- 1 vessel with transfer buffer for wetting transfer papers
- 1 vessel with at least 96% ethanol for pre-wetting of the PVDF card
- 1 vessel with transfer buffer for equilibration of the PVDF card

Note: *Some ethanols have auto fluorescent properties which will lead to a high membrane background. Make sure that the ethanol used during transfer is not auto fluorescent.*

Precautions

WARNING

Always wear gloves, protective clothing, and protective glasses when handling gel cards, PVDF cards and other consumables supplied with Amersham WB system.

Overview illustration of transfer sandwich assembly

The illustration below shows an overview of the transfer sandwich assembly.

The table below briefly describes the process of preparing a transfer sandwich:

Stage	Description
1	The PVDF card is pre-wetted in ethanol and then equilibrated in transfer buffer.
2	One sponge is placed on the transfer holder bottom part (black lid).
3	A transfer paper is pre-wetted in transfer buffer and then placed in the transfer holder.
4	The gel frame including the gel with separated proteins is removed from the gel frame support and placed in the transfer holder.
5	The PVDF card is placed in the transfer holder.
6	The second transfer paper is pre-wetted in transfer buffer and then placed in the transfer holder.
7	Air bubbles are removed by using the built in roller in the transfer holder.
8	A second sponge is placed on the transfer paper.
9	The transfer holder is closed.

Prepare the PVDF card

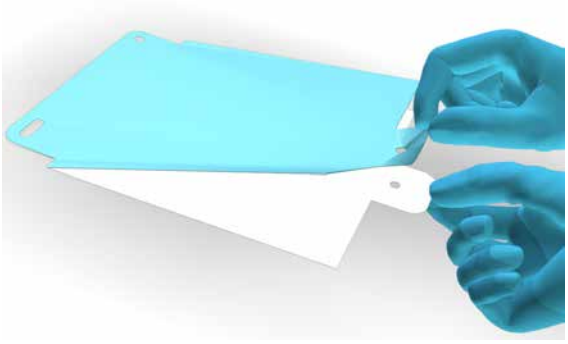
The instruction below describes how to prepare to build one transfer sandwich:

Step	Action
1	Take out one PVDF card single package from the PVDF card box.

Note:

Keep the package for the ID of the individual PVDF card and also for later storage.

2	Take out the PVDF card from the package and remove the first blue protective paper.

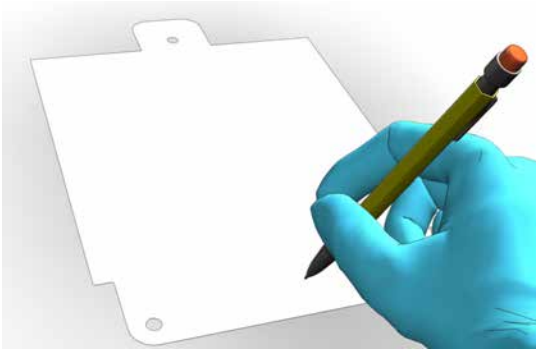


Step	Action
3	*Optional:*

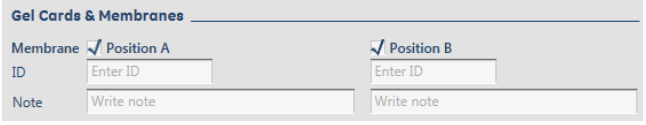
- Before removing the second blue protective paper, write an annotation (using a pencil) on the PVDF card writing surface.

  Tip:

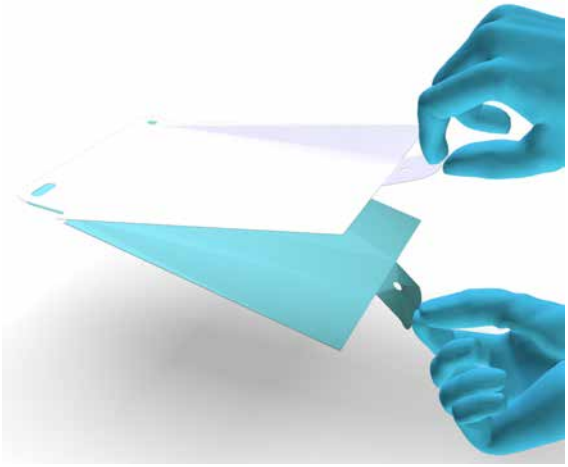
  The writing surface can be used to enter which position (A or B) where the PVDF card is used.



- Select the ***TRANSFER*** workflow step in the software. Put the cursor in the appropriate ***Note*** field and type in the text.

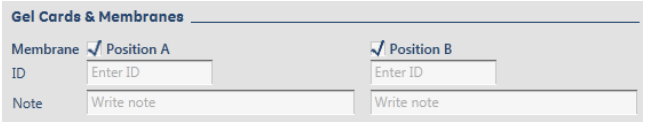


Step	Action
4	Remove the second blue protective paper.

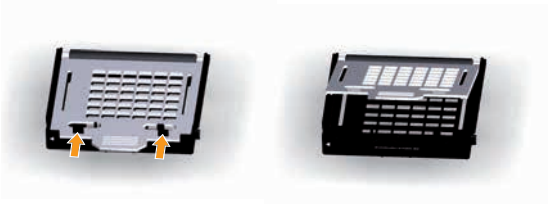


Note:

Do not touch the blotting area (marked with a red square in the picture) with your fingers. Hold the PVDF card using the handles.

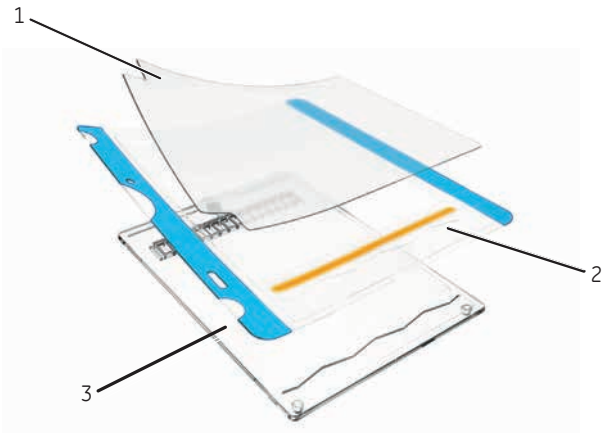
Step	Action
5	*Optional:* Enter PVDF card information in the software. • Select the ***TRANSFER*** workflow step in the software. • In the ***Gel Cards & Membranes*** area, place the cursor in the appropriate ***ID*** field. • Type in the identity number manually or scan the data matrix tag on the label of the PVDF card single package.  • To enter a note about the membrane, place the cursor in the ***Note*** field and type in a note. ***Tip:*** *Type in the lot number. This is not included in the data matrix but is available on the label on the box of PVDF cards.* 
6	Grip the PVDF card handles and pre-wet the card in ethanol by leaving it in the tray with ethanol for approximately 20 seconds.
7	Move the PVDF card to the PVDF card transfer buffer vessel and equilibrate for at least 5 minutes. ***Note:*** *Make sure that the PVDF card is covered with transfer buffer during this time.*

Start building the transfer sandwich

Step	Illustration of step	Operator actions
1		• Place the transfer holder with the black side facing downwards. • Open the transfer holder by pressing the latches and lifting the white lid.
2		• Push the lid until the roller pins reach the two cavities (marked with orange circles in the image). • Remove the white lid by lifting it through the two cavities.
3		Place the first sponge on the black lid.
4		Pre-wet one transfer paper in transfer buffer and place it on the sponge on the black lid (the transfer paper handle should be positioned between the guiding pins).

Overview illustration of the gel card disassembly

The illustration below shows an exploded view of the gel card parts to be disassembled after the electrophoresis run.

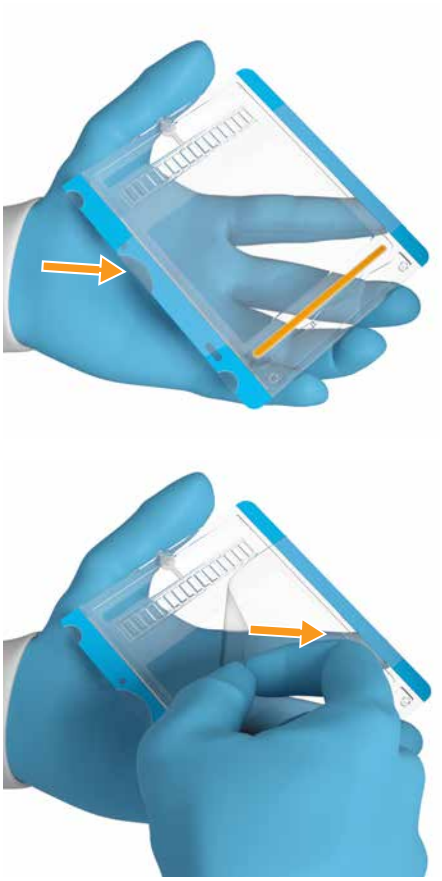


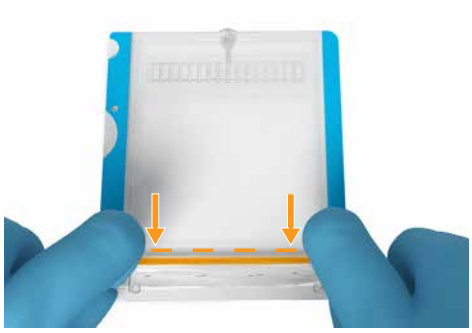
The table below briefly describes the parts of the gel card to be disassembled.

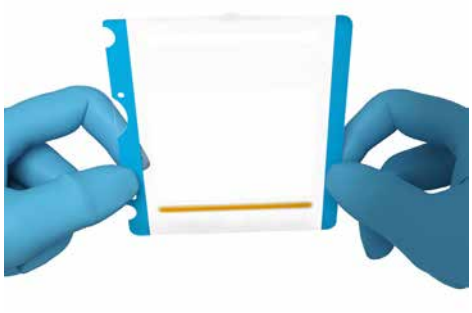
Stage	Description
1	Protective film. This film is removed first.
2	Gel frame The gel frame edges are pre-loosened from the gel frame support (3 in illustration) and then carefully removed.
3	Gel frame support stays on bench.

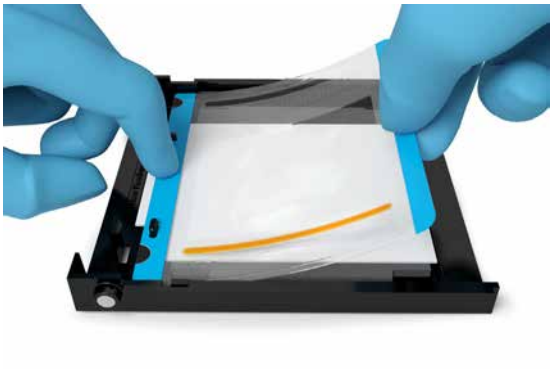
Loosen the gel frame from the gel frame support and place it in the transfer holder

Note: *Do not touch the gel area (marked with red cross) when loosening the gel frame.*

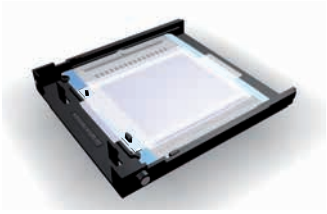
Step	Illustration of step	Operator actions
1		Hold the gel card with a steady grip. Remove the protective film by gripping the end indicated with an orange arrow and pulling it **diagonally** in the direction of the arrow.

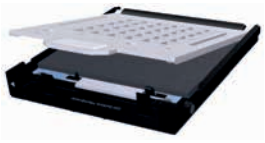
Step	Illustration of step	Operator actions
2		Place the gel card on the bench (with the exposed gel facing up) as illustrated in the image.
3		Grip the corners of the lower gel frame and loosen the gel frame between the lower corners as far as indicated by the dotted line and the arrows. ***Note:*** *When loosening the gel, continue to press the gel card towards the bench with your fingers.*
4		Grip the corners of the upper gel frame and loosen the gel frame as far as indicated by the dotted line. Make sure the gel frame is completely loosened from the white rubber plug (indicated by the upper orange arrow).

Step	Illustration of step	Operator actions
5	 	Continue to carefully loosen the gel frame from the edges of the gel card, in the direction towards the lower end, until the gel frame is completely loosened. ***Note:*** *When loosening the gel, continue to press the gel card towards the bench with your fingers.* ***Note:*** *Be careful not to touch the gel.*
6		Grip the gel frame handles and carefully lift the gel from the frame support.

Step	Illustration of step	Operator actions
7		Place the gel frame on the transfer paper by fitting the gel frame guiding holes onto the guiding pins and rolling the gel down on the transfer paper.

Place the PVDF card and finish building the transfer sandwich

Step	Illustration of step	Operator actions
1		Grip both the PVDF card handles with your fingers, or with help of flat tip tweezers. Remove the card from the transfer buffer tray. ***Note:*** *Do not touch the blotting area of the PVDF card (marked with a red square in picture).*
2		Place the PVDF card on the gel card by fitting the PVDF card guiding holes onto the guiding pins and rolling the PVDF card down on the gel. ***Note:*** *Do not adjust the placement of the PVDF card once placed on the gel card.*
3		Pre-wet one transfer paper in transfer buffer and place it on the PVDF card (the transfer paper handle should be positioned between the guiding pins).
4		Remove any air bubbles as follows: • Take the white lid and insert it into the two cavities (left image). • Grip the transfer paper handles with your left hand and drag the roller in the holder tracks to the other side of the transfer holder using your right hand.

Step	Illustration of step	Operator actions
5		Place the second sponge on the transfer paper.
6	 	Close the transfer holder by pressing the lid until the latches snap into place.

4.6.3 Run transfer

Introduction

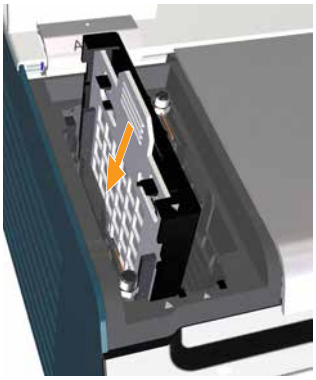
This section describes:

- how to load the transfer holder into the transfer tank
- how to start and monitor transfer
- the procedures to be performed after transfer

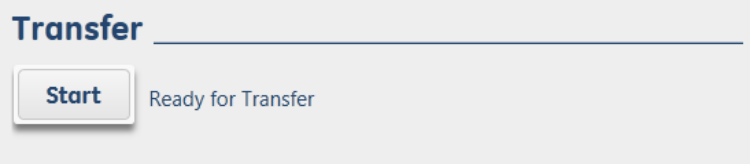
Before transfer

Make sure that transfer buffer and ultra pure water for the transfer run has been connected. See *Connect tubing to the transfer buffer and ultra pure water on the bottle rack, on page 185* for information.

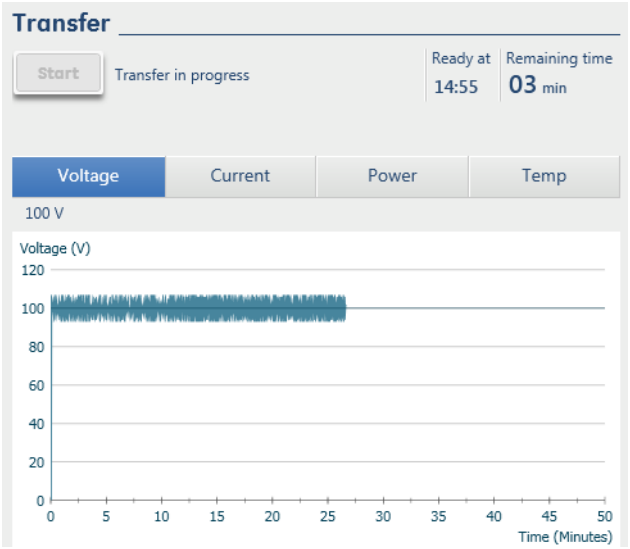
Load the transfer sandwiches

Step	Illustration of step	Operator actions
1		Open the transfer tank lid.
2		Insert the transfer holders into the tank. Make sure that the white arrow is pointing towards the arrow in the instrument (towards you).
3		Close the transfer tank lid.

Start and monitor transfer

Step	Action
1	Make sure that: • the **T buffer** and **T Water** tubing are immersed in the transfer buffer and ultra pure water bottles • the prepared transfer sandwiches have been loaded into the transfer tank in correct positions
2	In the ***TRANSFER*** workflow step in the software, click ***Start*** in the ***Transfer*** area.  *Result:* The transfer is started. The transfer tank indicator lamp on the Western unit changes to a blue triangle. ***Note:*** *During transfer, it is recommended to prepare and connect the solutions to be used in the probing step.* *See Section 4.6.5 Prepare and connect probing and antibody solutions, on page 208 for more information.*

Step	Action
3	The progress of the transfer is viewed in the ***Transfer*** area and graphs.



- **Graphs**

  By default, the graph shows the current for the progressing run.

  Click a tab (***Voltage***, ***Current***, ***Power***, ***Temp***) to view the corresponding graph for that parameter and the progress of the run.

- **Remaining time**

  The ***Remaining time*** for the run and when the run will be completed (***Ready at***) is displayed above the graph, to the right.

- **Run status**

  The status of the run is displayed to the right of the ***Start*** button. See *Section 2.9.5 Instrument status panel, on page 76* for information about the instrument status panel and status messages.

  During the run, the message ***Transfer in progress*** is displayed.

  When the run has finished the status to the right of the ***Start*** button is changed to ***Transfer completed***. The indicator lamp for the transfer tank on the Western unit changes to a white square.

Tip:

The progress of the run can also be viewed in the instrument status panel at the bottom of the screen. See Section 2.9.5 Instrument status panel, on page 76 for more information.

The name of the experiment, the remaining time and the run status are displayed.

Step	Action
	Note: *To abort a transfer run, select* ***Control:Abort run*** *in the menu bar. When a transfer has been aborted the protocol settings can be changed and the transfer restarted.*

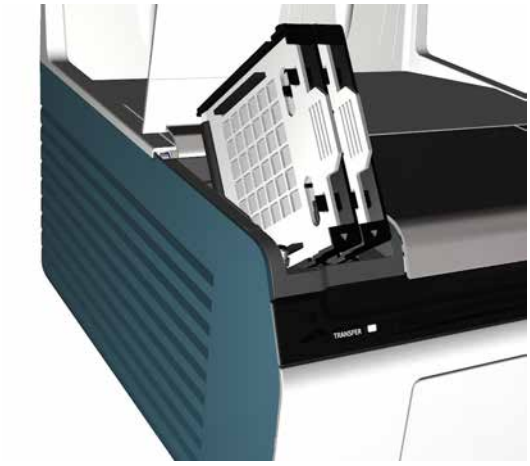
4.6.4 Procedures after transfer

Introduction

This section describes the cleaning procedures to be performed after transfer

Remove the transfer holder(s)

Step	Action
1	Open the transfer tank lid.
2	Remove the transfer holders. Hang them diagonally in the inner front of the transfer tank, above the liquid.



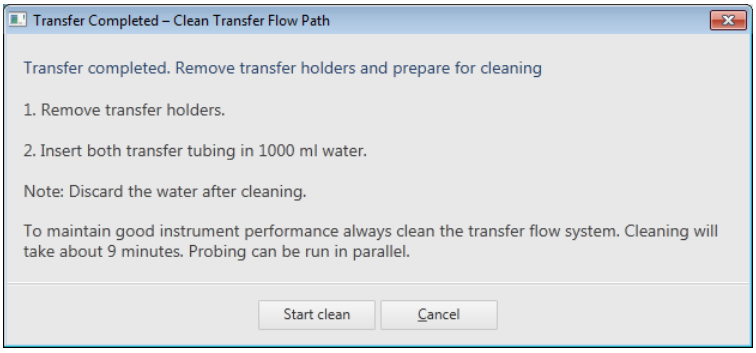
Note:

To avoid drying of membranes, do not open the transfer holders in this step.

Clean the transfer flow path

Note: *Before proceeding with cleaning, remove the transfer holder.*

Step	Action
1	When the transfer is completed, the ***Transfer Completed – Clean Transfer Flow Path*** dialog opens.



Note:

It is recommended to clean the transfer flow path after a run.

If you choose to skip cleaning of the transfer flow path, click ***Cancel*** *and proceed to Continue to probing, on page 207. However, remember to clean the transfer flow path at a later stage of the experiment. Cleaning can be performed by selecting* ***Control:Clean Transfer flow path*** *from the menu bar.*

If cleaning has been skipped the transfer flow path needs to be emptied manually by selecting, ***Empty Transfer tank*** *in the* ***Control*** *menu.*

2 Move the **T Buffer** tubing to the water bottle containing 1000 ml ultra pure water (together with the **T Water** tubing).

3 Click ***Start clean*** in the ***Transfer Completed – Clean Transfer Flow Path*** dialog.

The cleaning is started and will take approximately 9 minutes.

Note:

Probing can be started while cleaning the transfer flow path, see Continue to probing, on page 207.

4 Discard the solution in the water bottle and place the tubing in an empty bottle.

5 Wipe off the upper part of the transfer tank interior using a wet lint-free cloth.

6 Remove any remaining visual particles, fibers and gel pieces using tweezers.

Clean the transfer holder

WARNING

After electrophoresis, or after transfer (when the transfer sandwich has been opened and the PVDF card has been placed in the probing compartment), dispose of the gel in a safe way. Read the gel card Safety Data Sheet (SDS/MSDS) for safety instructions regarding the gel and disposal of the gel.

Note: *Perform this step when the transfer sandwich has been opened and the PVDF card has been placed in the probing chamber.*

Step	Action
1	Remove the gel from the transfer sandwich. Dispose of the gel according to instructions in the SDS/MSDS and local procedures for handling of waste.
2	Remove and discard the sponges and transfer papers.
3	Clean the different parts of the transfer holder using running water.
4	Leave the transfer holder to air dry.

Continue to probing

Do one of the following:

- If probing and antibody solutions have been prepared and connected to the Western unit, proceed with the instructions in *Section 4.6.6 Run probing, on page 211*

  else

- Proceed with *Section 4.6.5 Prepare and connect probing and antibody solutions, on page 208*

4.6.5 Prepare and connect probing and antibody solutions

Introduction

This section describes how to prepare and connect buffers and solutions for the probing run.

Probing and antibody solutions can be prepared during the transfer run and must be connected before starting the probing run.

Prepare probing and antibody solutions

See *Probing and antibody solution recipes, on page 104* for information about how to prepare solutions for the Western blot steps.

Connect tubing to the probing solutions on the bottle rack

CAUTION

To avoid the splashing of hazardous liquids, use heavy inlet filters connected to tubing in bottles.

See *Section 2.8 Other accessories, on page 61* for descriptions of inlet filters and tubing holders. See Section *Hardware installation* in *Amersham WB system Operating Instructions* for how to attach the accessories.

Before starting probing, connect tubing from the middle and right tubing towers to the solutions used in the probing run as follows:

Step	Action
1	From the middle tubing tower, place the tubing marked with: • **P Water** into a 1000 ml bottle with ultra pure water • **P Block** into a 100 ml bottle with blocking solution
2	From the right tubing tower, place the tubing marked with: • **P Wash** into a 1000 ml bottle with wash solution (PBS-T) • **P Final Wash** into a 500 ml bottle with final wash solution (PBS)

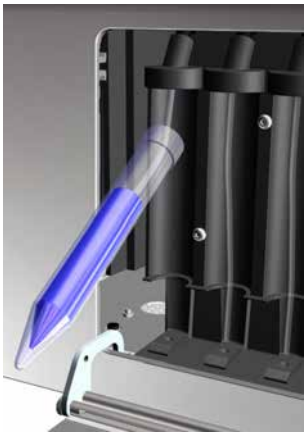
Step	Action
3	Before starting probing, connect the antibody solution tubes in the antibody compartment (see below for information).

Connect the antibody solution tubes

Note: *The antibody solutions should be prepared and connected close to the start of the probing.*

Step	Action
1	Open the antibody compartment on the Western unit by pressing the eject symbol on the antibody compartment lid.

Step	Action
2	Insert each antibody solution tube into the appropriate position, by placing the tubing in the tube and snapping the tube into position.



The table below describes which antibodies should be placed in which antibody tube position in the antibody compartment.

Position	Description
A PRIMARY	Position to be used for primary antibodies directed to membrane in position A in the probing chamber.
A SECONDARY	Position to be used for secondary antibodies directed to membrane in position A in the probing chamber.
B PRIMARY	Position to be used for primary antibodies directed to membrane in position B in the probing chamber.
B SECONDARY	Position to be used for secondary antibodies directed to membrane in position B in the probing chamber.

3	Close the antibody compartment lid.

Note: *For each membrane there is one antibody tube for the primary antibodies and one for the secondary antibodies. This means, for example, if two primary antibodies are selected for labeling of the membrane in* ***Position A****, they are mixed together in the same antibody tube and placed in position* ***A PRIMARY****.*

4.6.6 Run probing

Introduction

This section describes how to:

- place the PVDF cards in the Probing chambers
- start and monitor probing

Precautions

WARNING

After electrophoresis, or after transfer (when the transfer sandwich has been opened and the PVDF card has been placed in the probing compartment), dispose of the gel in a safe way. Read the gel card Safety Data Sheet (SDS/MSDS) for safety instructions regarding the gel and disposal of the gel.

CAUTION

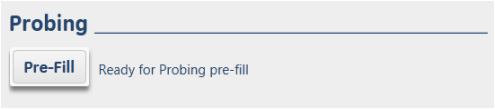
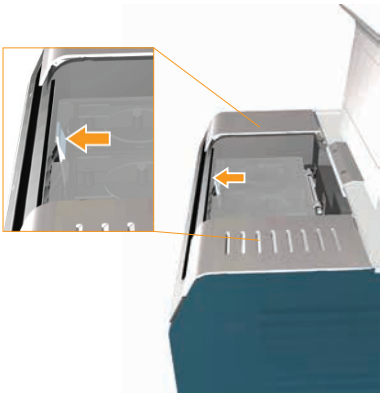
Pinch hazard. Do not touch the moving probing chamber during a run.

Before probing

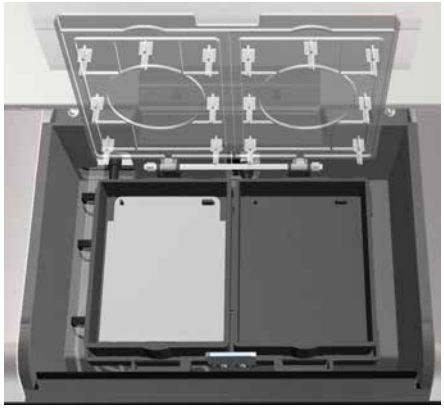
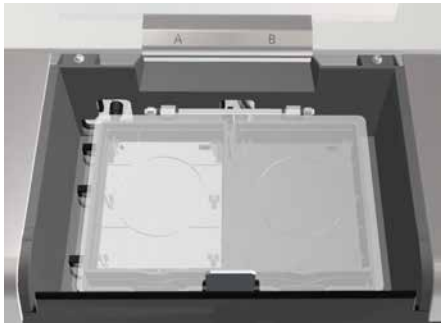
Make sure that buffers, ultra pure water, antibody, blocking and wash solutions are connected before starting probing.

Note: *Ultra pure water must always be connected, because it is used for rinsing between probing steps. Wash solution is used for rinsing between primary antibodies to position **A** and **B**.*

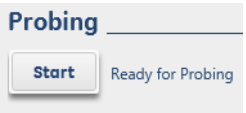
Pre-fill the probing chamber and load the PVDF cards

Step	Illustration of step	Operator actions
1	 	Pre-fill the probing chamber: • Click the ***PROBING & DRYING*** workflow step in the software. • Click ***Pre-Fill*** in the ***Probing*** area. *Result:* The probing chamber is filled with a few milliliters of the first solution in the probing sequence table (by default the blocking solution). ***Note:*** *After the probing chamber has been pre-filled:* • *It is no longer possible to change the type of the first step in the probing sequence table. However,* ***Dur. [min]*** *and* ***Repeat*** *can be edited.* • *The* ***Pre-Fill*** *button changes to a* ***Start*** *button and the status message* ***Ready for Probing*** *is displayed.*
2		Open the probing compartment lid and use the latch to open the probing chamber lid.

Step	Illustration of step	Operator actions
3		Open the transfer holder and remove the sponge and the transfer paper on top of the PVDF card.
4		• Remove the PVDF card from the transfer holder. Hold the PVDF card handles with your fingers, or use flat tip tweezers. • Place the PVDF card in the probing chamber, see step 5. • For cleaning the transfer holder and handling of waste, see *Clean the transfer holder, on page 207*.

Step	Illustration of step	Operator actions
5	 	Place the PVDF card in the pre-filled probing chamber by fitting the PVDF card holes over the guiding pins (marked with orange circles) and rolling the PVDF card down in the probing chamber. ***Note:*** *Take care not to touch the blotting area of the PVDF card.*
6	 	Close the probing chamber lid and the probing compartment lid. Make sure that the probing chamber lid is locked by the latch.

Start and monitor probing

Step	Action
1	Make sure that: • the probing tubing is immersed in the correct bottles for the probing run • the antibody solution tubes are connected in the antibody compartment
2	Click ***Start*** in the ***Probing*** area.  *Result:* Probing is started and the probing sequence table is locked for editing. The indicator lamp for the probing chamber on the Western unit changes to a blue triangle.

Step	Action
3	The probing progress is shown in the progress bar.

Completed steps in the probing sequence table are dimmed and the step currently running is also displayed in the instrument status area.

The ***Remaining time*** and when probing will be completed (***Ready at***) are displayed in the ***Probing*** control area.

When probing has been completed the status to the right of the ***Start*** button changes to ***Probing completed***. The indicator lamp for the probing chamber on the Western unit changes to a white square.

Tip:

The progress of the run can also be viewed in the instrument status panel at the bottom of the screen. See Section 2.9.5 Instrument status panel, on page 76 for more information.

The name of the experiment, the remaining time and the run status are displayed.

Note:

To abort a probing run, select ***Control:Abort run****. When probing has been aborted the protocol settings can be changed and the probing can be restarted. The probing will be restarted from the first step in the probing sequence.*

To re-start probing at the step where it was aborted, remove the completed probing steps and restart probing.

Step	Action
4	*Recovery of antibodies (if included in the setup):* Primary antibodies are automatically collected at the end of probing. When the ***Recover Primary Antibody solutions*** dialog opens: • Take out the antibody solution tubes with the recovered primary antibody solutions. • Click ***OK***.

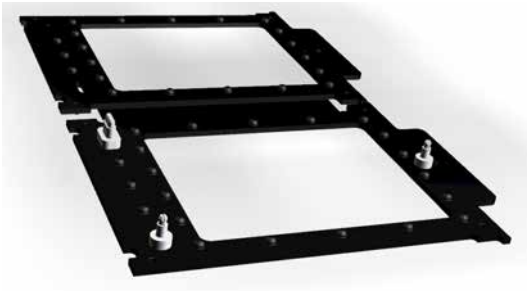
4.6.7 Procedures after probing

Introduction

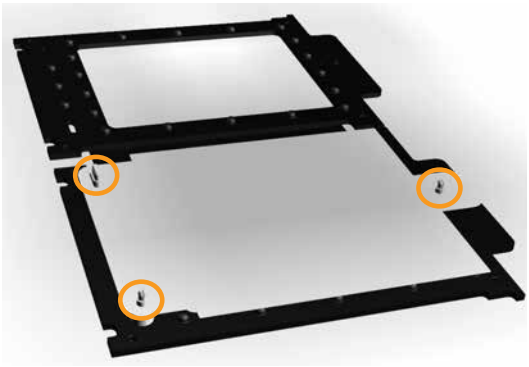
This section describes how to:

- place the PVDF card in the drying holder
- place the drying holder in the drying compartment and start drying
- clean the probing flow path

Move the PVDF card(s) to the drying compartment

Step	Action
1	Remove one Amersham WB drying holder (drying holder) from the drying compartment and open it. 
2	Grip the PVDF card handles with your fingers, or use flat tip tweezers, and lift the PVDF card out of the probing chamber.

Step	Action
3	Place the PVDF card in the drying holder by fitting the PDVF card holes over the three guiding pins.



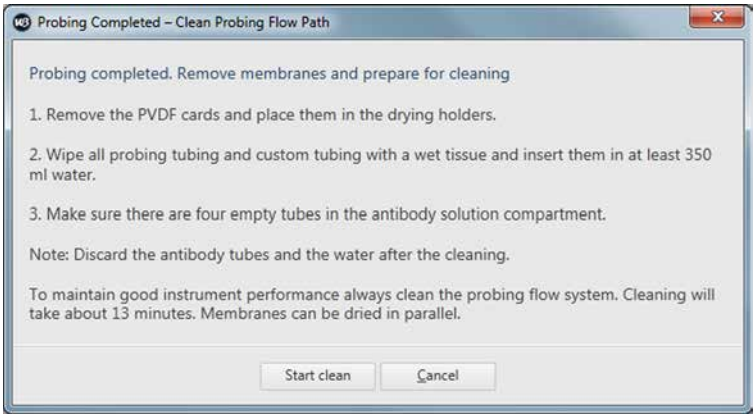
4	Close the drying holder. Make sure that the drying holder is locked by the catch.

5	Place the drying holder(s) in the appropriate position in the drying compartment (i.e., PVDF card in probing position **A** to drying position **A**).

Clean the probing flow path

Note: *Before proceeding with cleaning, move the PVDF card(s) to the drying compartment (see instructions above).*

Step	Action
1	When the probing is completed, the ***Probing Completed – Clean Probing Flow Path*** dialog opens.



Note:

It is recommended to clean the probing flow path after a run.

If you choose to skip cleaning of the probing flow path, click ***Cancel*** *and proceed to Section 4.6.8 Run drying, on page 222. However, remember to clean the probing flow path at a later stage of the experiment. Cleaning can be performed by selecting* ***Control:Clean Probing flow path*** *from the menu bar.*

If cleaning has been skipped the Probing flow path needs to be emptied manually by selecting, ***Empty Probing chamber*** *in the* ***Control*** *menu.*

2 Insert new empty antibody solution tubes in the antibody compartment.

Note:

Always insert ***four*** *new empty tubes.*

3 Wipe off the probing tubing **P Block**, **P Wash** and **P Final Wash** using a wet tissue and move the tubing to the bottle with ultra pure water (containing the **P Water** tubing).

In the software dialog check the amount of ultra pure water needed for cleaning, make sure that the bottle contain at least that amount of water.

Step	Action
4	Click ***Start clean*** in the ***Probing completed – clean probing system*** dialog. The cleaning is started and will take approximately 13 min. ***Note:*** *Drying of membranes can be started while cleaning the probing flow path.*
5	When cleaning is completed, discard the solution in the bottle with water, and discard the waste water in the antibody solution tubes. Place the tubing in an empty bottle.

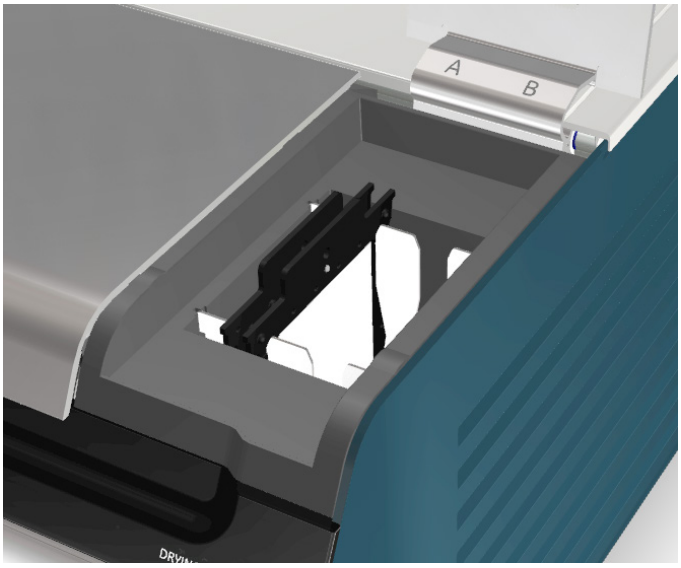
4.6.8 Run drying

Introduction

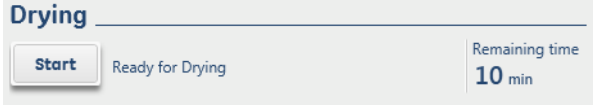
This section describes how to perform drying of PDVF cards. Drying the PVDF cards will give an even and low background, and stronger singals.

Start and monitor drying

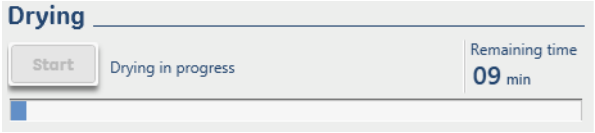
Step	Action
1	Make sure that the drying holders with PVDF cards have been placed in the drying compartment in the correct positions (i.e., PVDF card in probing position **A** is placed in drying position **A**).



2	Click ***Start*** in the ***Drying*** area.



Result: Drying is started. The indicator lamp for the Drying compartment on the Western unit changes to a blue triangle.

Step	Action
3	The drying progress is shown in the progress bar and the status message displays ***Drying in progress***. The ***Remaining time*** is displayed in the ***Drying*** area.  ***Note:*** *To abort a drying run, select* ***Control:Abort run****. The drying can be restarted after it has been aborted.*
4	When the run has finished, the status to the right of the ***Start*** button is changed to ***Drying completed***. The indicator lamp for the Drying compartment on the Western unit changes to a white square. The card is ready for scanning. See *Section 4.6.9 Scan membrane and view results, on page 224* for information. ***Tip:*** *The drying progress run can also be viewed in the instrument status panel at the bottom of the screen. The name of the experiment, the remaining time and the run status is displayed.* ***Note:*** *If the membranes are not completely dry, click* ***Start*** *in the* ***Drying*** *area to perform a new drying run.*

4.6.9 Scan membrane and view results

Introduction

This section describes how to:

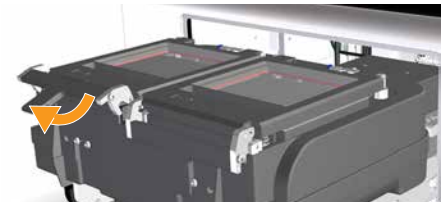
- place the PVDF card in the Elpho & scan unit
- start and monitor scanning
- view the results of the scan

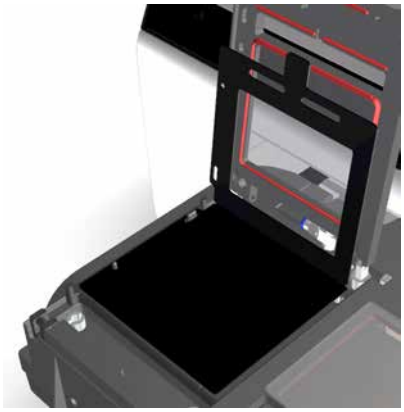
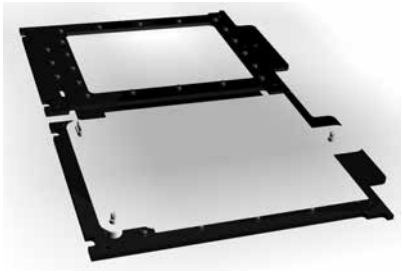
Place PVDF card in the loader

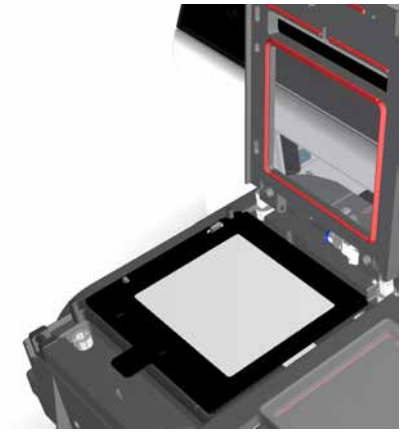
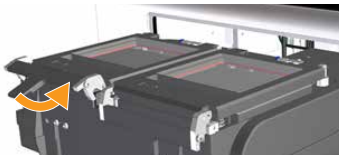
CAUTION

Moving parts. Be careful when opening/closing the loader to avoid fingers or clothing becoming trapped when the loader is moving.

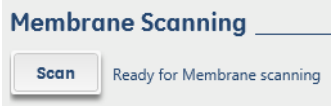
Never place any bottles or vials in front of the Elpho & scan unit. They may fall when the loader is opened.

Step	Illustration of step	Operator actions
1		Press the eject button on the Elpho & scan unit.  *Result:* The loader is ejected.
2		• Turn the latch upwards and open the sealing lid. • Wipe off any liquid and dirt from the card plate protective glass using a lint-free cloth. It is important to keep the protective glass clean in order to obtain a good result when scanning gel cards and PVDF cards.

Step	Illustration of step	Operator actions
3		• Remove a membrane adapter from the antibody compartment of the Western unit. ***Note:*** *Make sure the membrane adapter is dry and clean.* • Open the membrane adapter and place it on the card plate by fitting the guiding holes in the membrane adapter over the guiding pins. ***Note:*** *Be careful not to scratch the card plate with any sharp objects.*
4		Open the drying holder.
5		Remove the PVDF card from the drying holder, and place the card in the membrane adapter by fitting the guiding holes over the guiding pins. ***Note:*** *Take care not to touch the blotting area of the PVDF card.*

Step	Illustration of step	Operator actions
6		Close the membrane adapter.
7		Close the sealing lid.
8		Press the eject button on the Elpho & scan unit. *Result:* The loader is closed.

Start and monitor membrane scanning

Step	Action
1	Make sure that the PVDF card(s) is placed in the membrane adapter(s) on the card plate(s) and the loader is closed.
2	Click ***Scan*** in the ***Membrane Scanning*** area.  *Result:* Membrane scanning is started. The indicator lamp on the Elpho & scan unit changes to a blue triangle.

Step	Action
3	Viewing the progressing scan:

- The progress of the scans are displayed in the ***Membrane Scanning*** area.
- The status message to the right of the ***Scan*** button is changed to ***Scanning membranes***.
- The ***Remaining time*** is displayed in the ***Membrane Scanning*** area.

Note:

To abort a scanning, select ***Control:Abort run****. When scanning has been aborted the scanning protocol can be modified and the scan restarted. The aborted image is included in the image stack.*

Step	Action
4	When the scanning has been completed, the membrane images will be displayed in the ***Membrane Scanning*** area.

If two colors (Cy5 and Cy3) were used in the experiment, two images, one for each color, will be displayed for each position.

The status message changes to ***Membrane scanning completed***. The indicator lamp on the Elpho & scan unit changes to a white square.

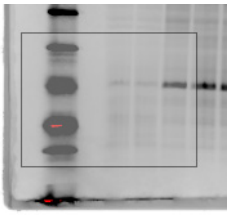
Overview - membrane scanning result

When membrane scanning has been completed, the scanned membrane images are displayed in the ***Membrane Scanning*** area.

If two colors have been used, one image/color (Cy3 and Cy5 channels) are displayed. If one color has been used, one image is displayed.

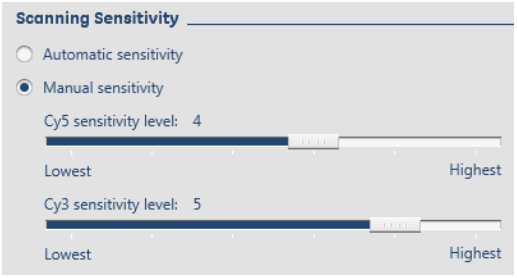
For information about how to zoom out of the image, adjust contrast and brightness, display image information, export an image and turn annotations on or off, see *Tool descriptions – membrane scanning, on page 233*.

Assess the image quality

Step	Action
1	Check the scanned images. It is possible to zoom in on the image by holding down the left mouse button and dragging a rectangle over the membrane area of interest. Saturated signals in the membrane images are displayed in red (annotations must be turned on, see *Tool descriptions – membrane scanning, on page 233*).  • If you have saturated signals in the areas of interest of your membrane images, consider re-scanning your membranes using a lower sensitivity level. It is possible to calculate volumes, but the volumes of saturated areas should not be used for quantitative purposes. • If you have too weak signals in the areas of interest of your membrane images, consider re-scanning your membrane images using a higher sensitivity level.
2	If you need to re-scan your images, see *Re-scan of membrane images, on page 231* for instructions.
3	If the images look fine, see *Procedures after membrane scanning, on page 235* and then proceed with evaluation of the experiment described in *Chapter 5 Evaluate an experiment, on page 237*.

Re-scan of membrane images

Step	Action
1	In the ***Scanning Sensitivity*** area, select the ***Manual sensitivity*** radio button.



- Drag the Cy5 sensitivity slider to the appropriate sensitivity level to change sensitivity in the Cy5 images scanning.
- Drag the Cy3 sensitivity slider to the appropriate sensitivity level to change sensitivity in the Cy3 images scanning.

Note:

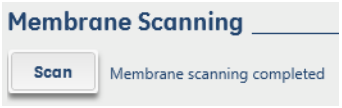
When comparing different membranes it is important to use the same sensitivity settings when scanning the membranes.

Tip:

The sensitivity level used at scanning for the currently displayed membrane image can be viewed by clicking the Image information tool.

If you have several scanned images, the sensitivity level for each image can also be viewed by clicking the Image stack tool.

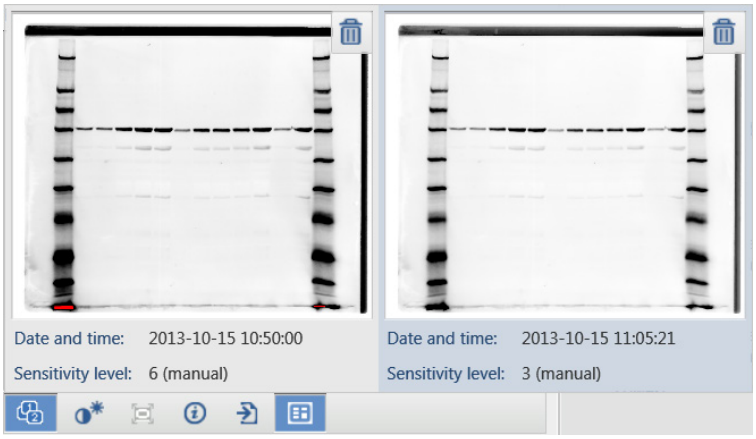
2 Click ***Scan*** to start scanning the membranes with the new settings.



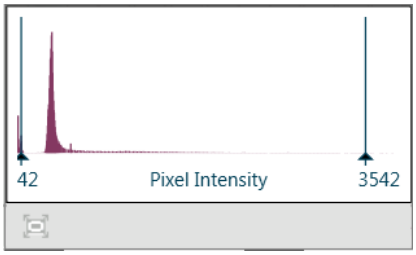
Note:

To abort a scanning, select ***Control:Abort run****. When scanning has been aborted the scanning protocol can be modified and the scan restarted. The aborted image is included in the image stack.*

3 When the scanning has been completed, the latest scanned images are displayed in the ***Membrane Scanning*** area.

Step	Action
4	It is possible to compare the latest scanned membrane image with previously scanned membrane images by clicking the Image stack tool. Up to four images can be contained in the stack.  A window with all scanned images is displayed showing the ***Date and time*** and ***Sensitivity level*** used at scanning, and if automatic or manual sensitivity was used. 
5	Choose the best membrane image by clicking on the appropriate image. *Result:* The selected image is displayed in the ***Membrane Scanning*** area. This image will be passed on to the ***EVALUATION*** workflow step.
6	Proceed with the ***EVALUATION*** workflow step to evaluate your results. See *Chapter 5 Evaluate an experiment, on page 237* for information about how to evaluate your results.

Tool descriptions – membrane scanning

Tool	Description
	Image stack tool. Click to display different scans of the same membrane image. • To delete an image from the image stack, click the Delete symbol at the top right corner of the image to be removed. • To select an image to display in the ***Membrane Image Preview***, click the appropriate image. ***Note:*** *Four images per membrane can be kept in the image stack at the same time.*
	Contrast and brightness tool. Click to open the contrast and brightness control.  You can directly modify the brightness and contrast of the display by dragging the handles on the graph. • Moving the handles closer together increases the contrast (and vice versa) of the pixels within the range. See *Contrast and brightness, on page 235* for more information. • To reset any changes, click the  tool in the contrast and brightness control window. The change does not alter any raw data or calculations within the software. It is only a view setting, and optimum values are actually dependent on the current monitor.

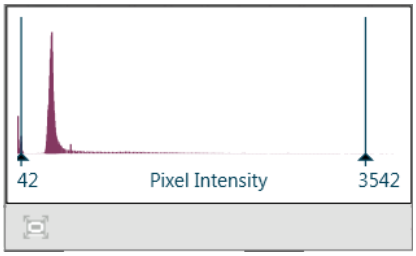
Tool	Description
	Zoom out tool. This tool is only enabled when an image has been zoomed in on. Click to fully zoom out of the image. ***Tip:*** *It is possible to zoom out by double clicking in the image.* ***Tip:*** *Zoom in on an image by holding down the left mouse button and dragging a rectangle over the membrane area of interest. It is possible to zoom in several times in an image.*
	Image information tool. Click to display the ***Image information*** dialog. It shows data about the image currently displayed in the ***Membrane Scanning*** area.
	Export image tool. Select to export the image as: • Normal export (.tif), or • Compressed export (.jpg) In the ***Export image*** dialog browse to the appropriate folder, type in a name for the image and click ***Save***. ***Note:*** *When exporting to *.tif format, the contrast of the image may need to be adjusted before export in order to obtain the correct appearance in the software used (TIFF has over 65000 intensity levels and a normal screen 256).* ***Tip:*** *It is also possible to copy an image to the clipboard by right-clicking on the membrane image and selecting **Copy** (or press the **Ctrl+C** on the keyboard) if you do not to save the image as a file. The membrane image can then be pasted into another application.*
	Annotations tool. Click to turn on/off the annotations in the membrane images. If the tool is blue it means that annotations are turned on. If the tool is white it means that annotations are turned off.

Contrast and brightness

The contrast and brightness control displays the frequency with which each pixel intensity occurs within the image. The peaks on the graph represent the pixel intensities that occur most frequently within the image.

The left and right handles on the graph show the range of pixel intensities in the image that will be mapped to a gray scale in the display image. Pixels with intensities below the left handle will be displayed as completely white in the image. Pixels with intensities above the right handle will be displayed as completely black in the image. Pixels between the handles will be displayed in various shades of gray.

The raw image can use up to 65536 intensity levels. The display normally displays 256 levels. The contrast and brightness translate between the wide range raw image and the display.



For information about how to change contrast and brightness, see *Tool descriptions – membrane scanning, on page 233*.

Procedures after membrane scanning

Step	Action
1	Press the eject button on the Elpho & scan unit. *Result:* The loader is ejected.
2	Open the sealing lid.
3	Remove the membrane adapter with the PVDF card from the card plate.
4	Remove the PVDF card from the membrane adapter. ***Tip:*** *The PVDF card can be stored in a dried condition between two filter papers in the original PVDF card single package. Signals on the dried PVDF card are stable for about 3 months.*

Step	Action
5	Clean the membrane adapters, using a lint-free cloth, and return the adapters to the storage space in the antibody compartment.
6	Clean the drying holders using a lint-free cloth, and return the holders to the drying compartment.
7	Wipe off the card plate, using a lint-free cloth, if needed wetted with 50% ethanol.
8	Wipe off the protective glass, using a lint-free cloth, if needed wetted with 50% ethanol. ***Note:*** *It is important to keep the protective glass clean on both sides in order to obtain a good result when scanning gel cards and PVDF cards.*
9	Close the sealing lid and press the eject button on the Elpho & scan unit. *Result:* The loader is inserted.

5 Evaluate an experiment

About this chapter

This chapter describes how to evaluate an experiment.

In this chapter

This chapter contains the following sections:

5.1 Introduction

Introduction

The evaluation of Image analysis data is described in this chapter as a suggested workflow.

An automatic evaluation is performed, followed by a check of the result and editing of analysis settings, if necessary. Different analysis approaches depending on the experiment type are briefly described. Detailed descriptions and instructions for result tabs are found in a separate section. Finally, there is a section describing how to edit the analysis settings used if errors were found when results were checked.

Images from the A and B positions in the experiment are evaluated independently.

Workflow overview

Stage	Description
1	Perform evaluation in Amersham WB software.
2	Check the result of the analysis settings used (Lane detection, Band detection, Band matching and MW calibration) If necessary, edit analysis settings.
3	Analyze the results for the experiment type and export the results.

Note: *The workflow is repeated for both positions A and B if both positions are used in the experiment. Tabs for results for position A and and B are displayed in the first display shown.*

5.2 Perform evaluation and check results

Introduction

This section describes:

- how to evaluate the results of an experiment
- the default result views for different experiment types
- how to check that the analysis settings used during evaluation give the appropriate results
- how to copy and export experiment results

For information about how to analyze a specific experiment type, see *Section 5.3 Analyze the results of an experiment type, on page 247*.

Evaluate an Amersham WB experiment

Step	Action
1	In Amersham WB software, open the experiment.
2	Click the ***EVALUATION*** workflow step in the workflow panel to the left. *Result:* Default view (image and result) dependent on the experiment type is displayed.

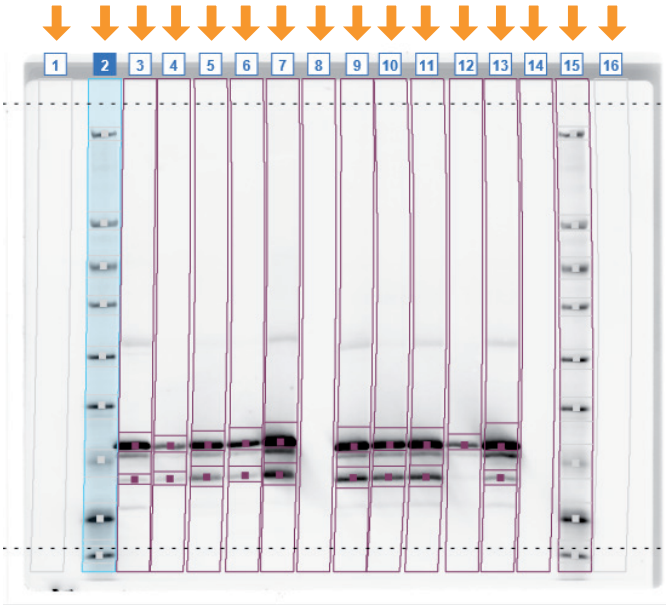
Default result views

The table below describes the default result views for the different experiment types.

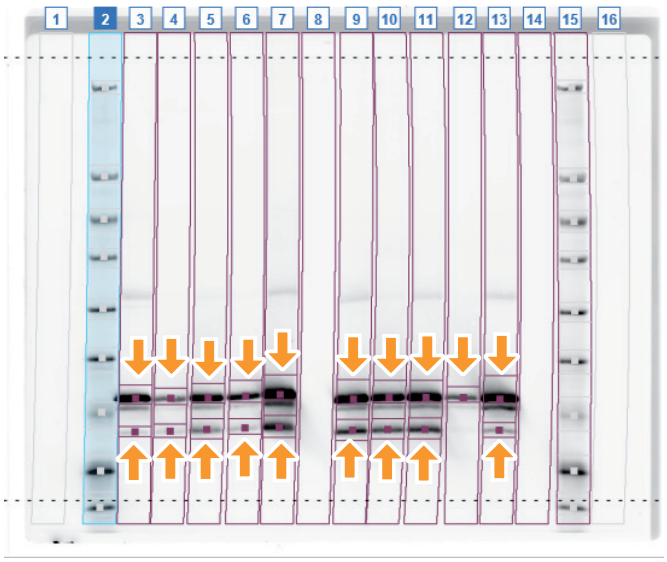
Experiment type	Default view
Easy Western	• Membrane image for Primary antibody 1 with detected and matched protein bands • Protein table showing Volume
Western with total protein normalization	• Membrane image Cy3 (target) with detected protein bands • Bar chart showing Normalized ratios

Experiment type	Default view
Western with endogenous protein normalization	• Membrane images overlay with detected protein bands. Green bands are Cy3 and red bands are Cy5. • Bar chart showing Normalized ratios
Easy SDS-PAGE	• Gel Cy5 image with detected protein bands • Lane profile of the first lane set to MW marker, Sample or Quantity calibrant
Easy SDS-PAGE with quantity calibration	• Gel Cy5 image with used quantity calibrants annotated • Quantity calibration curve
Easy Western with quantity calibration	• Membrane image for Primary antibody 1 with used quantity calibrants annotated • Quantity calibration curve

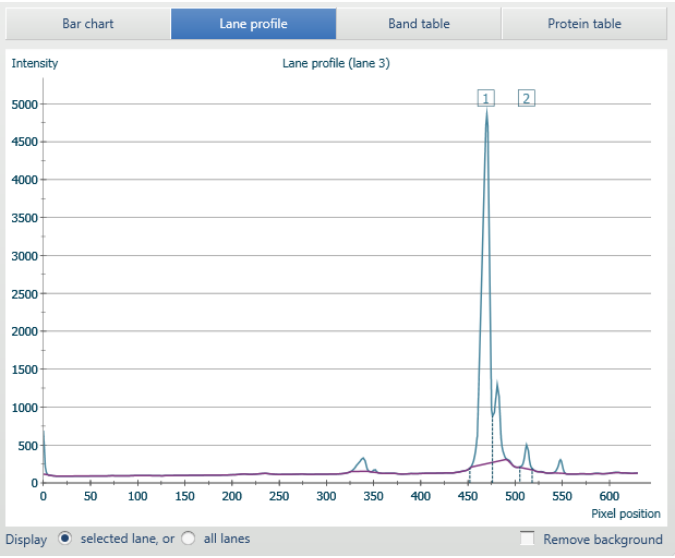
Check the results of the analysis settings used

Step	Action
1	**Check lane detection**. Check that all lanes are detected accurately. Lanes are detected individually for the gel image and for the Cy3/Cy5 membrane images the lane detection is common.  To edit the lane detection, see *Section 5.5.1 Edit lane detection, on page 292*. ***Note:*** *By clicking the **Default** button in **Detect lanes** tab when in Edit mode, the experiment is re-evaluated with the current band parameters, that is, the band detection, matching and molecular weight are also re-calculated. Therefore, editing of lanes should be performed first and before editing band detection and bad matching etc.*

Step	Action
2	**Check band detection**. Check if band detection is satisfactory. In the displayed gel/membrane image, detected bands are annotated with purple squares.

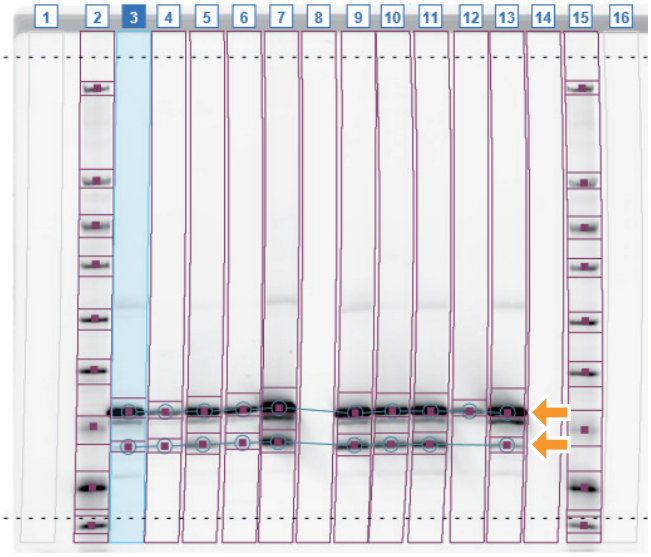


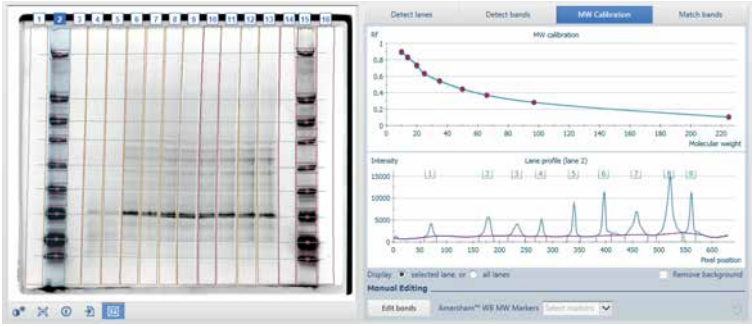
Step	Action
3	Click the ***Lane profile*** tab to display the lane profile of a selected lane. Detected bands have numbered boxes above each peak.



A band can be selected by clicking on either the band in the image or the band number in the lane profile. Selected bands are highlighted both in the image (yellow box) and in the lane profile (peak number becomes blue).

To edit the band detection, see *Section 5.5.2 Edit band detection, on page 297*.

Step	Action
4	**Check band matching.** • Click ***Protein table*** tab and check matching of detected bands. Matched bands are annotated with blue circles connected with lines. • The matching of bands is relevant in the Protein table. Matched bands are placed in the same row in the table.  To edit the band matching, see *Section 5.5.3 Edit band matching, on page 304*

Step	Action
5	**Check Molecular weight (MW) calibration.** • Click ***Edit***. • Click ***MW Calibration*** tab. *Result:*  • Select a lane used for MW calibration and check in the Lane profile that correct bands have been detected as MW calibrants. • Repeat the procedure for all lanes used for MW calibration. • Check that the MW calibration curves are OK. • Check that the green lines between markers in the image view are horizontal. These are used for MW calibration for the lanes between the MW marker lanes. For a description of the MW calibration algorithm, see *Description of the molecular weight calibration algorithm, on page 315* ***Note:*** *If only one lane is used for MW calibration, all lanes have been MW calibrated using the curve of that lane.* • If MW calibration has failed, an error message is shown between the MW calibration curve and the Lane profile To edit the MW calibration, see *Section 5.5.4 Edit molecular weight calibration, on page 309*

Analyze the results for an experiment type

For the different experiment types, default views differ and different result formats (tables and charts) are used. Also, the analysis procedures differ between experiment types. See *Section 5.3 Analyze the results of an experiment type, on page 247* for more information about the different analysis procedures.

Copy and export experiment results

Objective	Action
Copy image views, bar charts and tables	• Right-click in the Image view and select ***Copy image***, or • Right-click in a Bar chart and select ***Copy graph***, or • Right-click in a table and select ***Copy table*** ***Note:*** *When copying a table, only the columns that are currently displayed in the table are copied to the clipboard. Non-rounded off figures are copied.* Paste the copied object into a document (e.g., Microsoft Excel or Word).
Export data from a bar chart or table	• Right-click in the graph or table and select ***Export.*** • Browse for a folder, enter a name and click ***Save.*** The format is a text file (.txt). • To open the the file in for example, Notepad, double-click the file, or • To open the file in Microsoft Excel, select the file, right-click and select ***Open with.*** Choose Microsoft Excel. ***Note:*** *When exporting a table all available columns are exported to the file regardless of if they are currently displayed or not. Non-rounded off figures are exported.*

5.3 Analyze the results of an experiment type

Introduction

For the different experiment types, default result views differ and different result formats (tables and charts) are used. Also, the analysis procedures differ between experiment types.

In this section some suggested experiment analysis procedures are described.

For a description of each result tab, independent of the experiment type, see *Section 5.4 Result views in Amersham WB software, on page 262*

In this section

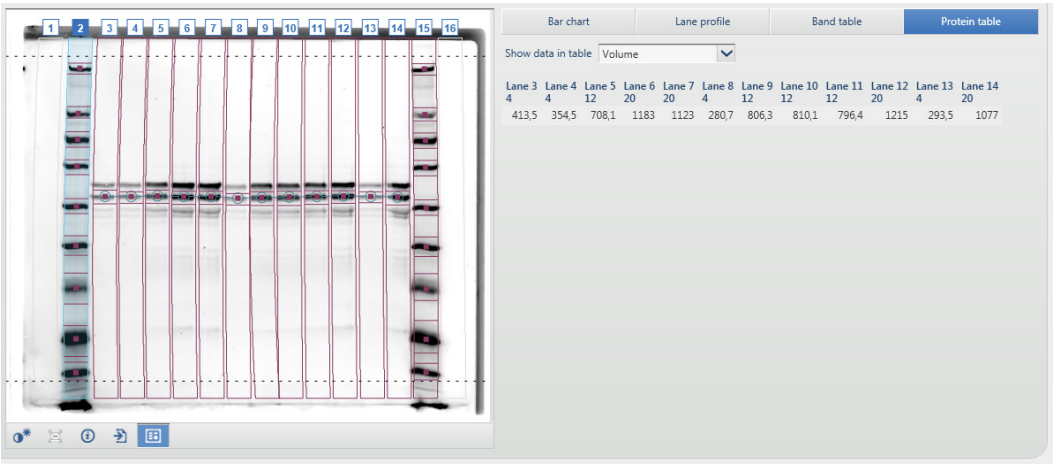
This section contains the following subsections:

5.3.1 Analyze an Easy Western experiment

Default result view

- Membrane image for Primary antibody 1 with detected and matched protein bands.
- Protein table showing Volume.

Example of result view



Analysis procedure

Objective	Action	Reference for detailed information
Visual analysis	Analyze the displayed membrane view. Detected bands are annotated with a purple square and matched bands with circles and connected lines.	See *Section 5.4.1 Image view, on page 263*
Analysis of calculated data	Analyze the calculated data in the displayed Protein table. Matched bands are shown in the same row.	See *Section 5.4.6 The Protein table tab, on page 288*

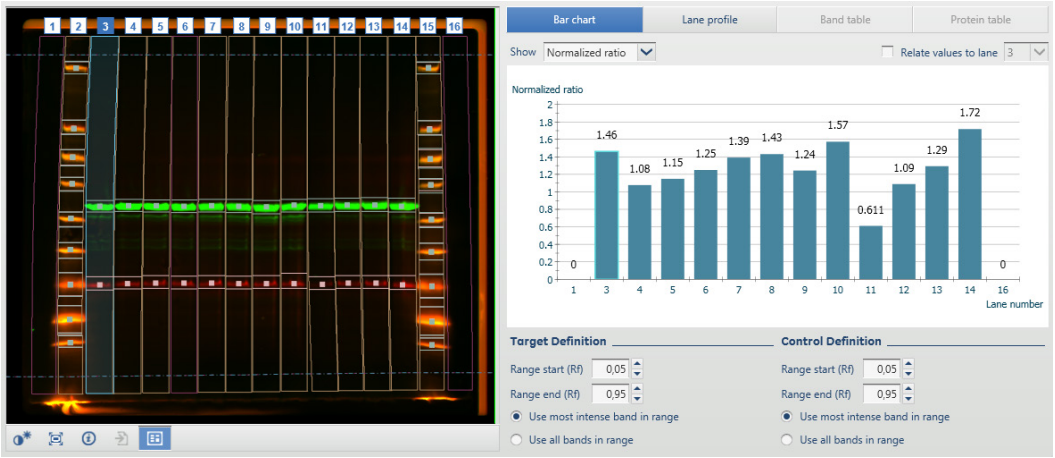
Objective	Action	Reference for detailed information
Analysis of Image view and calculated data for a Primary antibody 2	If a primary antibody 2 has been used in the experiment, select to display the other Cy channel. Analyze the displayed membrane view and calculated data in the displayed protein table.	See *Section 5.4.6 The Protein table tab, on page 288*
Analysis of ratios of signals for Primary antibody1 and Primary antibody 2	Click the ***Bar chart*** tab and select to show the ratio of Cy signals.	See *Section 5.4.2 The Bar chart tab, on page 272*
Copy and export experiment results		See *Copy and export experiment results, on page 246*

5.3.2 Analyze a Western experiment with endogenous protein normalization

Default result view

- View of Membrane Overlay with detected protein bands. Green bands are Cy3 (in this example Target) and red bands are Cy5 (in this example Control)
- Bar chart showing Normalized ratio

Example of result view



Analysis procedure

Objective	Action	Reference for detailed information
Visual analysis of the image view	Analyze the displayed Membrane overlay. Green bands are Cy3 and red bands are Cy5. Turquoise dotted lines, are displayed for Cy3 range and dark-red dotted lines are displayed for Cy5 range.	See *Section 5.4.1 Image view, on page 263*

Objective	Action	Reference for detailed information
Define target and control	Define range and usage of bands for target and control. See the ***Target Definition*** and ***Control Definition*** areas below the Bar chart. ***Note:*** *Select to show individual membranes (Control or Target) for a better view.*	See *Section 5.4.2 The Bar chart tab, on page 272*
Analyze normalized ratios in the bar chart	Click the ***Bar chart*** tab. The normalized ratio is calculated as: Volume $_{Target}$/Volume $_{Control}$, both with subtracted background.	See *Section 5.4.2 The Bar chart tab, on page 272*
Copy and export experiment results		See *Copy and export experiment results, on page 246*

5.3.3 Analyze a Western experiment with total protein normalization

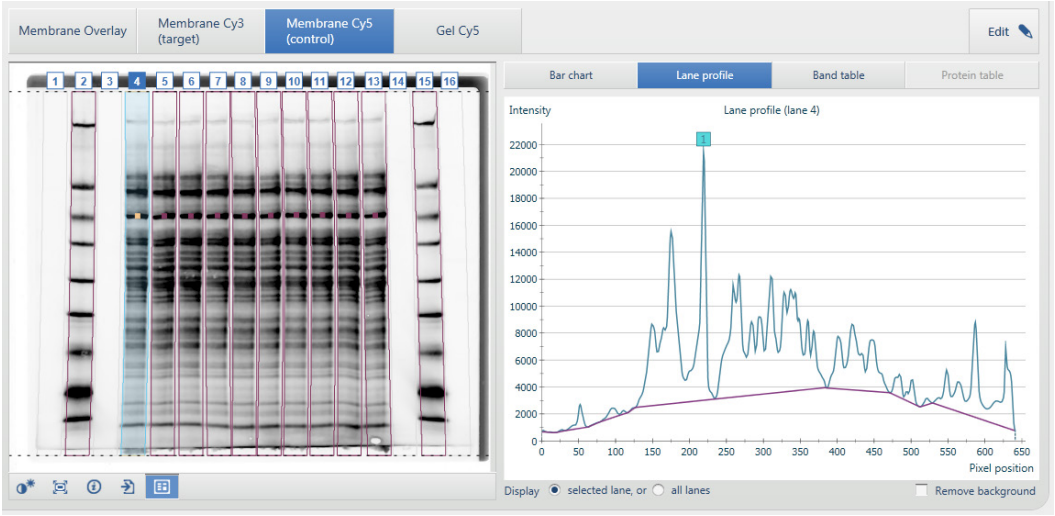
Default result view

- View of Membrane Cy3 (target) with detected protein band
- Bar chart showing Normalized ratio

Examples of result views

Membrane Overlay

Membrane Cy5 (control)



Analysis procedure

Objective	Action	Reference for detailed information
Visual analysis of target membrane image	Analyze the displayed Membrane image.	See *Section 5.4.1 Image view, on page 263*
Define target	Define range and usage of bands for target . See the ***Target Definition*** area below the Bar chart. ***Note:*** *Select to show individual membrane (Target) for a better view.*	See *Section 5.4.2 The Bar chart tab, on page 272*
Analyze control membrane image	Click the ***Membrane Cy5 (control)*** tab. The Volume of the control is calculated as one band. The background is calculated using a defined rolling ball radius. This may be edited by the user. See illustration above, *Example: Membrane Cy5 (control)*	See *Section 5.4.1 Image view, on page 263*

Objective	Action	Reference for detailed information
Analyze normalized ratios in the bar chart	Click the ***Bar chart*** tab. The normalized ratio is calculated as: Volume $_{Target}$/Volume $_{Control}$, both with subtracted background.	See *Section 5.4.2 The Bar chart tab, on page 272*
Copy and export experiment results		See *Copy and export experiment results, on page 246*

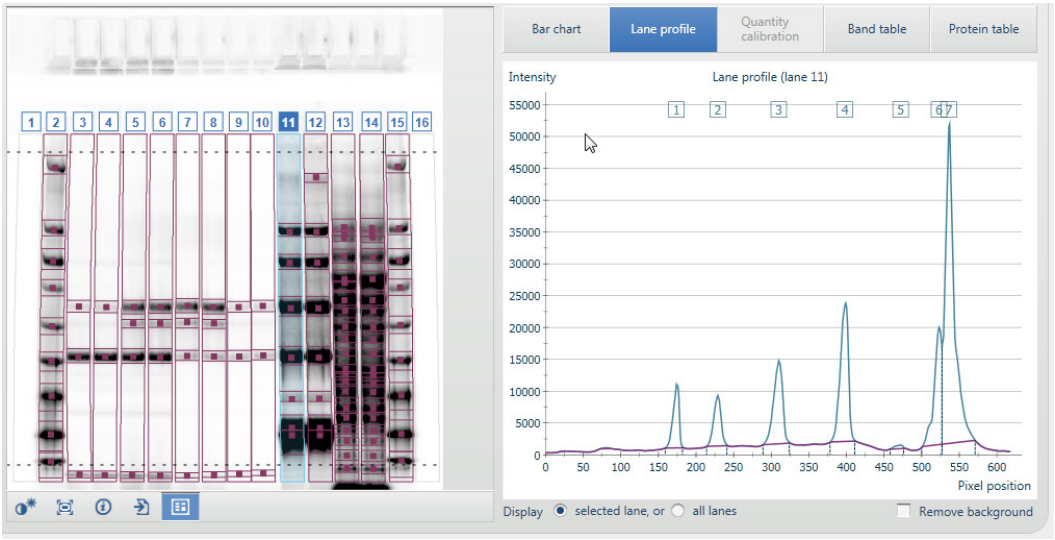
5.3.4 Analyze an Easy SDS-PAGE experiment

Default result view

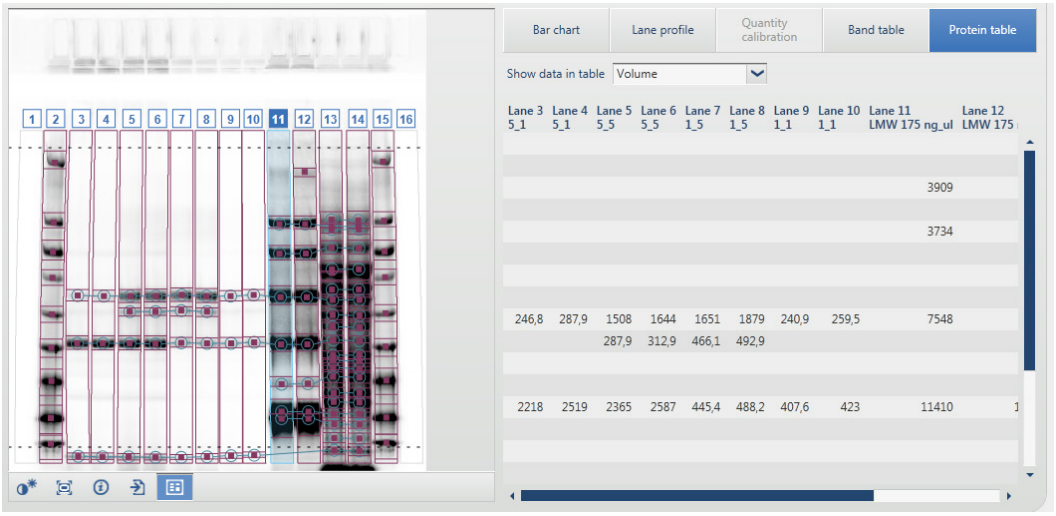
- Gel Cy5 (visualization of separation of pre-labelled proteins)
- Lane profile of the first lane set to MW marker, Sample or Quantity calibrant

Examples of result views

Gel view and Lane profile



Gel view and Protein table



Analysis procedure

Objective	Action	Reference for detailed information
Visual analysis of gel view	Perform visual analysis of separated protein samples.	See *Section 5.4.1 Image view, on page 263*
Analysis of data in Protein table	To display the Protein table, click the ***Protein table*** tab.	See *Section 5.4.6 The Protein table tab, on page 288*
Analysis of molecular weights	In the protein table, select to show MW.	See*Section 5.4.6 The Protein table tab, on page 288*
Purity check of a sample	By looking at the Band% data in the Protein table for a detected band (corresponding to the protein to be purified) within a lane, the purity of a sample containing that protein can be estimated.	See *Section 5.4.6 The Protein table tab, on page 288*

Objective	Action	Reference for detailed information
Fraction analysis	By looking at the Volume or Band% data in the Protein table for a detected band in different lanes, the concentration or purity of a sample between fractions can be followed	See *Section 5.4.6 The Protein table tab, on page 288*
Copy and export experiment results		See *Copy and export experiment results, on page 246*

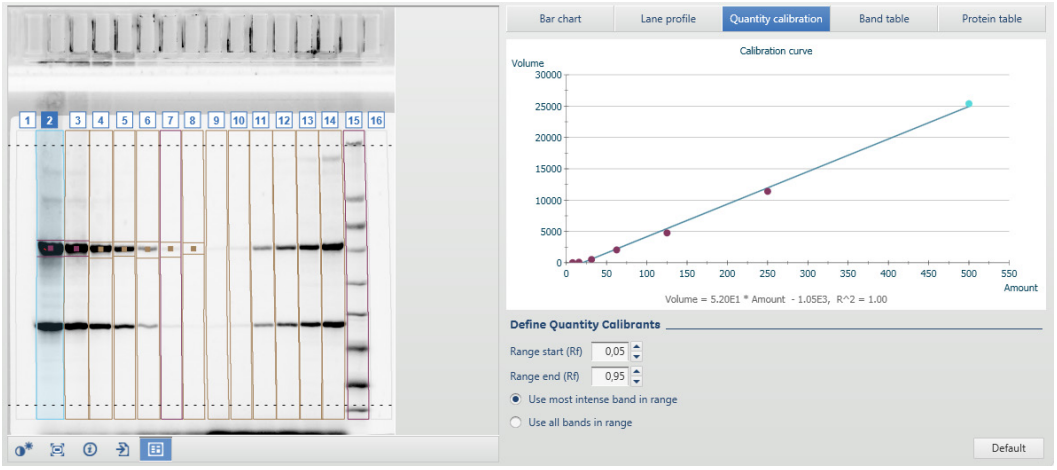
5.3.5 Analyze an Easy SDS-PAGE experiment with quantity calibration

Default result view

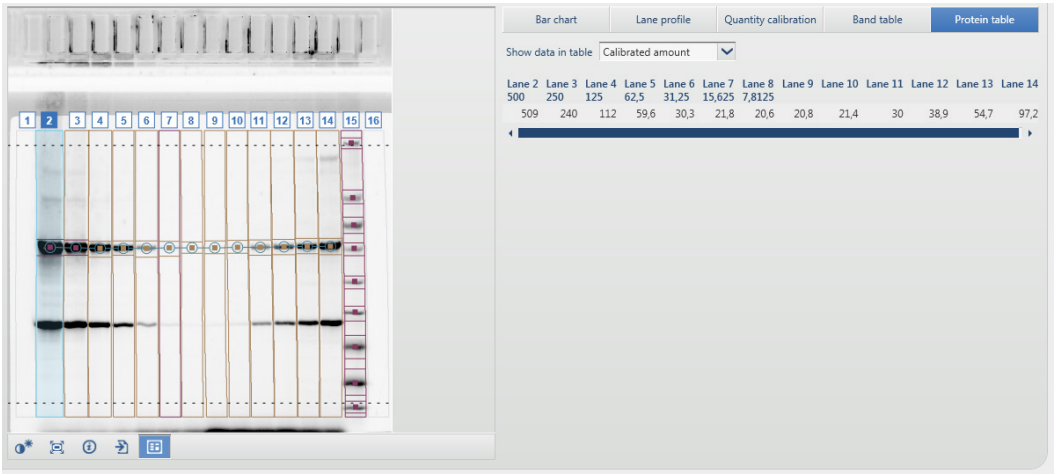
- Gel Cy5 (Visualization of separation of pre-labelled proteins)
- Calibration curve

Examples of result views

Gel view and Calibration curve



Gel view and Protein table



Analysis procedure

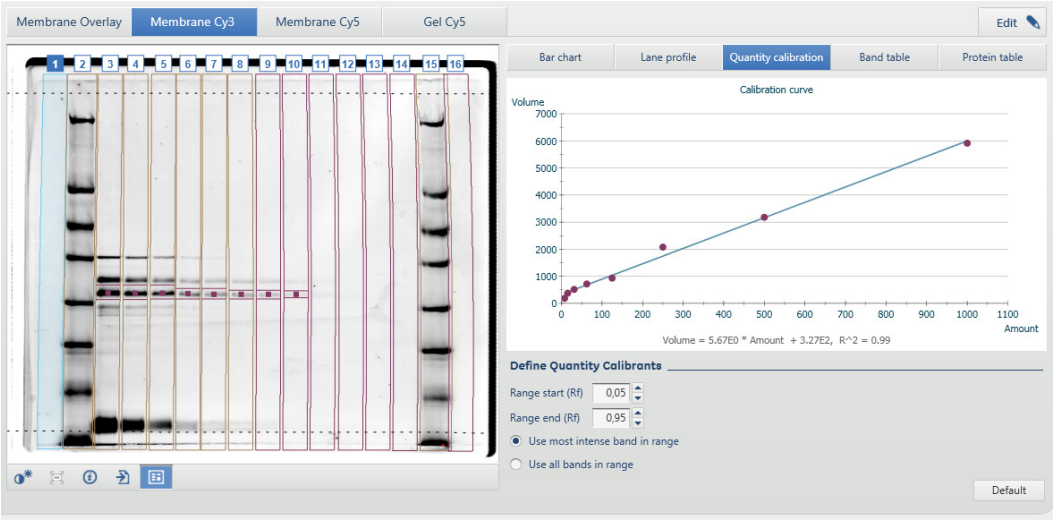
Objective	Action	Reference for detailed information
Visual analysis of the Calibration curve	Analyze the curve and the displayed curve data below the curve (the equation and the coefficient of regression). Bands used in the calculation of the Calibration curve are annotated with purple squares.	See *Section 5.4.5 The Quantity calibration tab, on page 285*
Define Quantity calibrants	Define range and usage of bands. See the ***Define Quantity calibrants*** area below the Quantity calibration curve.	See *Section 5.4.5 The Quantity calibration tab, on page 285*
Obtain calibrated amounts of all detected bands	Click the ***Protein table*** tab. Calibrated amount is also shown in the Band table.	See *Section 5.4.6 The Protein table tab, on page 288*
Copy and export experiment results		See *Copy and export experiment results, on page 246*

5.3.6 Analyze an Easy Western experiment with quantity calibration

Default result view

- Membrane image for Primary antibody 1 with detected and matched protein bands
- Calibration curve

Example of result view



Analysis procedure

Objective	Action	Reference for detailed information
Visual analysis of Calibration curve	Analyze the curve and the displayed curve data below the curve (the equation and the coefficient of regression). Bands used in the calculation of the Calibration curve are annotated with purple squares.	See *Section 5.4.5 The Quantity calibration tab, on page 285*

Objective	Action	Reference for detailed information
Define Quantity calibrants	Define range and usage of bands. See the ***Define Quantity calibrants*** area below the Calibration curve.	See *Section 5.4.5 The Quantity calibration tab, on page 285*
Obtain calibrated amounts of all detected bands	Click the ***Protein table*** tab. Calibrated amount is also shown in the Band table.	See *Section 5.4.6 The Protein table tab, on page 288*
Copy and export experiment results		See *Copy and export experiment results, on page 246*

5.4 Result views in Amersham WB software

Introduction

The displayed results for an experiment consist of the image view in combination with the:

- bar chart
- quantity calibration curve (where applicable)
- lane profile
- band table
- protein table

This section describes the Image view and the different result tabs.

For the different experiment types, default views differ and different result formats (tables and charts) are used. Also, the evaluation procedures differ between experiment types. For information about how to evaluate the results for a specific experiment type, see *Section 5.3 Analyze the results of an experiment type, on page 247*.

In this section

This section contains the following subsections:

5.4.1 Image view

Introduction

The Image view is displayed for all result tabs in the ***EVALUATION*** workflow step. This is used together with the information on the result tabs to view the results for an experiment.

This section describes the:

- image view
- image types that can be displayed in the Image view
- annotations in the Image view
- contrast and brightness tool (overview)
- tools that are used to adjust the appearance of the image view, display image information and export an image.

Image view overview

The image view is displayed for all tabs in the ***EVALUATION*** workflow step. It shows the image selected by choosing one of the tabs above the Image view (Membrane Overlay, Membrane Cy5/Cy3, Gel Cy5). Image annotations are displayed by default. Below the image view, tools for adjusting contrast and brightness, zoom out, view image information, export images and turn on/off annotations are available.

Depending on which experiment type was selected when creating the experiment, different image types are shown by default. See *Image types, on page 264* below for more information about the image types.

Image types

The following image types can be displayed for the different experiment types in the ***EVALUATION*** workflow step. For ***Easy SDS-PAGE*** experiments, only the gel image is available. For the Western experiments, depending on the experiment type and setup, up to four image types are available.

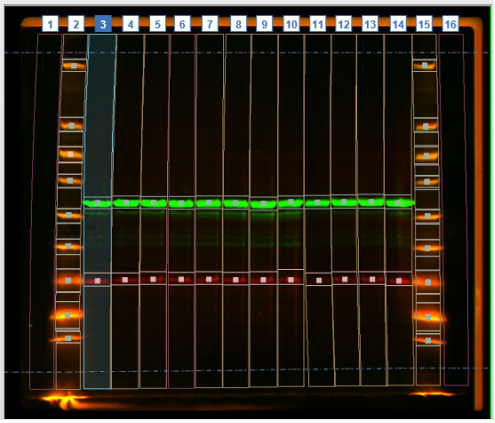
Image type	Description
Membrane Overlay 	This image is displayed as default for the experiment type ***Western with endogeneous protein normalization***. It shows an overlay of the Cy5 and Cy3 images. • Cy3 signals are shown in green. Cy3 band range definitions are shown as turquoise dotted lines. • Cy5 signals are shown in red. Cy5 band range definitions are shown as dark-red dotted lines. • Proteins visible in both channels, such as the molecular weight markers, are yellow because the marker proteins are labeled with both Cy3 and Cy5. ***Note:*** *In **Western with endogeneous protein normalization** experiments, Cy3 and Cy5 are not linked to target or control. See the **EXPERIMENT & SAMPLES** workflow step in the experiment to view the selection of secondary antibodies for the target and for the control.* *In **Western with total protein normalization**, Cy3 is used to detect the target and Cy5 is used to detect the control.*

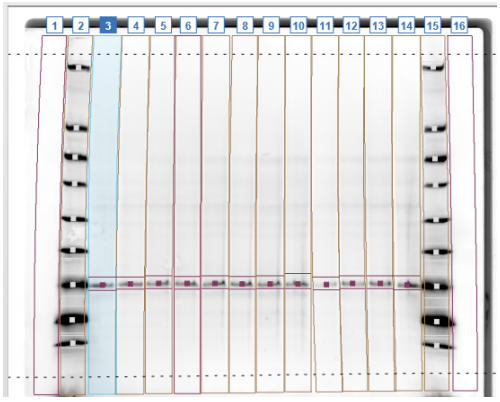
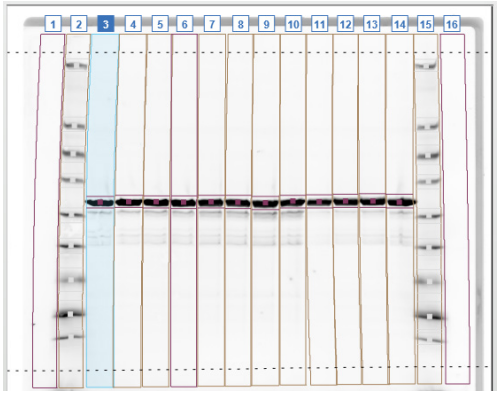
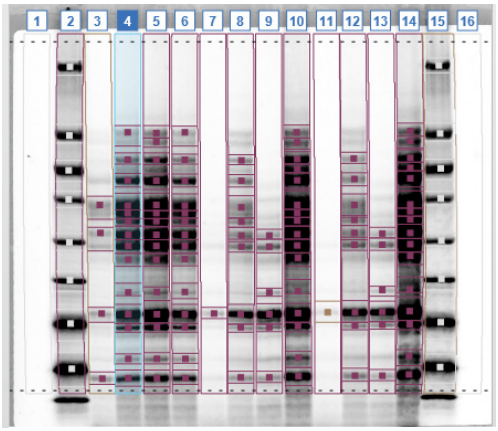
Image type	Description
First membrane image tab 	This image is displayed as follows: • For ***Easy Western*** experiments the tab is named ***Membrane Cy3*** if Cy3 has been selected to detect the first primary antibody and ***Membrane Cy5*** if Cy5 has been selected to detect the first primary antibody. • For ***Western with total protein normalization*** experiments the tab is displayed as default, and is named ***Membrane Cy3 (target)***. • For ***Western with endogenous protein normalization Membrane Cy3 (target)*** if Cy3 has been selected to detect the first primary antibody and ***Membrane Cy5 (target)*** if Cy5 has been selected to detect the first primary antibody.
Second membrane image 	This image is displayed as follows: • For ***Easy Western*** experiments the tab is named ***Membrane Cy3*** if Cy3 has been selected to detect the second primary antibody and ***Membrane Cy5*** if Cy5 has been selected to detect the second primary antibody. • For ***Western with total protein normalization*** experiments ***Membrane Cy5 (control)***. • For ***Western with endogenous protein normalization Membrane Cy3 (control)*** if Cy3 has been selected to detect the second primary antibody and ***Membrane Cy5 (control)*** if Cy5 has been selected to detect the second primary antibody.

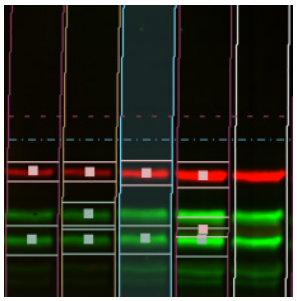
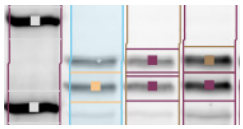
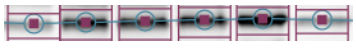
Image type	Description
Gel Cy5 	This image is displayed by default for the experiment type ***Easy-SDS PAGE***. It shows the Cy5 signals from pre-labeled samples on the gel image. The wells are included in the image (not shown).

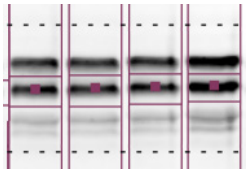
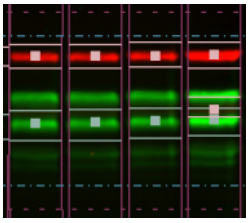
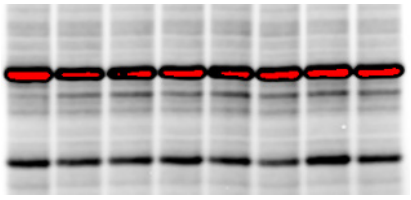
Annotations

Annotations are displayed in the Image view by default. Annotations can be turned on/off by clicking the Annotations tool below the Image view.

The table below describes what is annotated in the Image view.

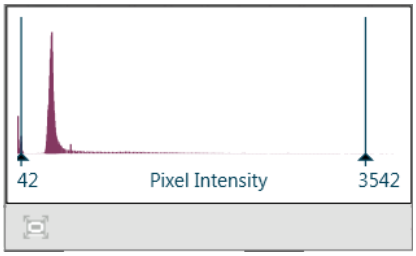
Annotation	Description
Well number	A well number is displayed in a box above each lane representing wells 1-16 in the gel card. • A lane number in a white box indicates a non-selected lane • A lane number in a blue box indicates a selected lane 

Annotation	Description
Lanes	Lines enclosing the lanes show the lane detection. • Purple lines represent non-edited lanes • Brown lines represent lanes that have been edited • Blue lines and a blue highlight of the lane represent selected lanes • White lines represent a lane that has been excluded in the Edit mode 
Bands	Detected bands are indicated by a square and a line above (band start) and a band below (band stop) the black Cy3 or Cy5 signal. • Purple square and lines represent a band • Yellow square and lines represent any selected band • Brown square and lines represent an added or edited band • White square and lines represent detected bands that are not used in the calculation for the currently displayed result If displaying the overlay image, green color represents Cy3 signals and red color Cy5 signals. Proteins visible in both channels, such as the molecular weight markers, are yellow because the marker proteins are labeled with both Cy3 and Cy5. 
Matched bands	Matched bands are visible when displaying the ***Protein table*** tab. Matched bands are surrounded by blue circles. A blue line connecting the matched bands is also displayed. 

Annotation	Description
Marker bands used in MW calibration	Green lines between each corresponding MW marker represent marker bands which are used for MW calibrating the bands in the lanes between the lanes with MW calibrants. 
Target/control definitions and quantity calibrant definitions	Dotted black lines across the image view.  If displaying the overlay image, the dotted target definition lines are turquoise and the dotted control definition lines are dark-red. 
Saturated signals	Saturated signals are shown as red. 

Tool descriptions

The tools below the Image view are used to adjust the image view display, show data about the image, and to copy or export the image. The table below describes the available tools.

Tool	Description
	Contrast and brightness tool. Click to open the contrast and brightness control.  Modify the brightness and contrast of the display by dragging the handles on the graph. • Moving the handles closer together increases the contrast (and vice versa) of the pixels within the range. See *Contrast and brightness overview, on page 271* for more information. • To reset any changes, click the  tool in the contrast and brightness control window. The change does not alter any raw data or calculations within the software. It is only a view setting, and optimum values are actually dependent on the current monitor. ***Note:*** *For the overlay image two contrast and brightness tools are displayed next to each other. One applies to the Cy3 image and the other to the Cy5 image. The contrast and brightness is thus adjusted for each of the images independently. The two separate histograms can be used to tune the appearance of the overlay image.*

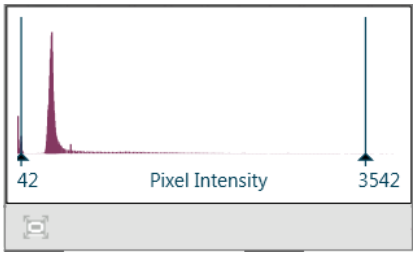
Tool	Description
	Zoom out tool. This tool is only enabled when an image has been zoomed. Click to fully zoom out of the image. ***Tip:*** *Zoom in on an image by holding down the left mouse button and dragging a rectangle over the gel area of interest. It is possible to zoom in several times in an image.* ***Tip:*** *To zoom out in steps, double-click in the image.*
	Image information tool. Click to to display the ***Image information*** dialog. It shows data about the image currently displayed in the Image view.
	Export image tool. Select to export the image as: • Normal export (.tif), or • Compressed export (.jpg) In the ***Export Image*** dialog browse to the appropriate folder, type in a name for the image, and click ***Save***. ***Note:*** *The export function is not available for **Membrane Overlay** images.* ***Note:*** *When exporting to *.tif format, the contrast of the image may need to be adjusted before export in order to obtain the correct appearance in the software used (TIFF has over 65000 intensity levels and a normal screen 256).* ***Tip:*** *It is also possible to copy the displayed image to the clipboard by right-clicking on the membrane image and selecting **Copy** (or press **Ctrl+C** on the keyboard). The image can then be pasted into another application.*
	Annotations tool. Click to turn on/off the annotations in the Image view. If the tool is blue it means that annotations are turned on. If the tool is white it means that annotations are turned off. See *Image view overview, on page 263* for more information about annotations.

Contrast and brightness overview

The contrast and brightness control displays the frequency with which each pixel intensity occurs within the image. The peaks on the graph represent the pixel intensities that occur most frequently within the image.

The left and right handles on the graph show the range of pixel intensities in the image that will be mapped to a gray scale in the display image. Pixels with intensities below the left handle will be displayed as completely white in the image. Pixels with intensities above the right handle will be displayed as completely black in the image. Pixels between the handles will be displayed in various shades of gray.

The raw image can use up to 65536 intensity levels. The display normally displays 256 levels. The contrast and brightness translate between the wide range raw image and the display.



For information about how to change contrast and brightness, see *Tool descriptions, on page 269*.

5.4.2 The Bar chart tab

Introduction

The bar chart tab is used to display bars and values of selected options for each lane.

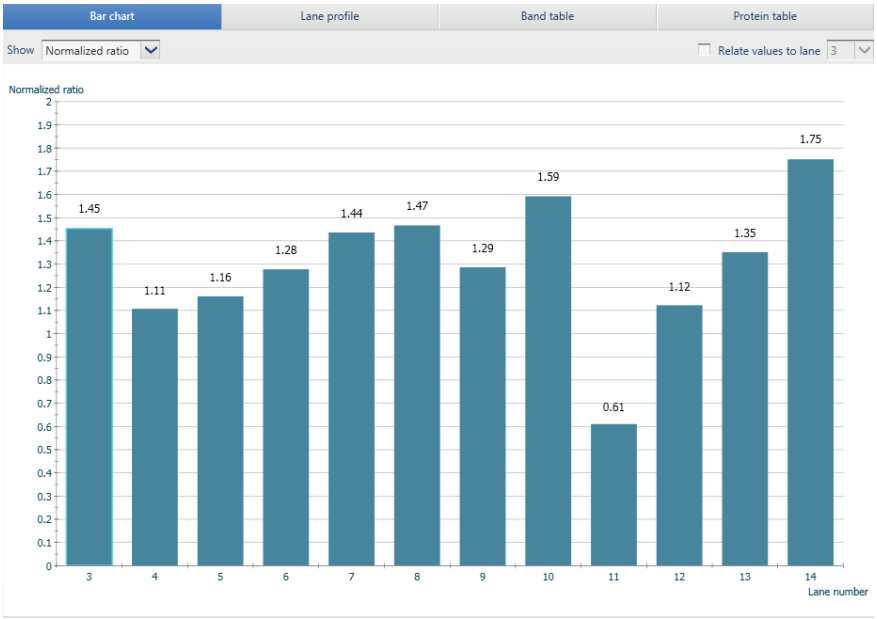
The tab is displayed as default for the following Experiment types where it is used to display normalized values.

- Western with endogenous protein normalization, and
- Western with total protein normalization

Western with normalization

Volumes of a defined target protein have been normalized using the:

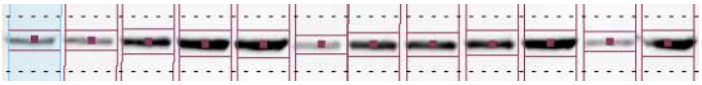
- volume for all proteins (***Western with total protein normalization***). Cy5 pre-labeled proteins are shown in the ***Membrane (control)*** view. All proteins are detected as one band.
- volume of a selected control protein (***Western with endogenous protein normalization***). Bands of the secondary antibody dye, specific to the used primary antibody bound to the endogenous protein, are shown in the ***Membrane (control)*** view.



Target and control range definitions

The target and control range definitions are displayed in the images in the Image view as dotted lines.

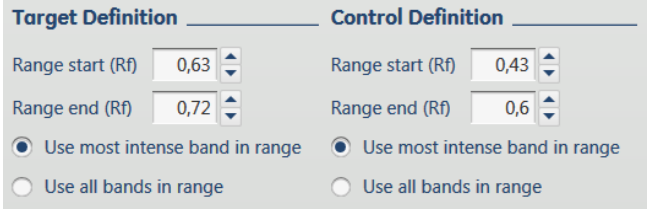
If the ***Membrane overlay*** is displayed, turquoise horizontal dotted lines, are displayed for Cy3 and dark-red horizontal dotted lines are displayed for Cy5 in the same image. The images in the ***Membrane overlay*** are green for Cy3 and red for Cy5, on a black background.



Adjust Target and Control definitions

The target range definition can be adjusted for all experiment types and the control range definition can be adjusted for ***Western with endogenous protein normalization***. The definitions define which bands that will be used in the calculation.

Note: *In **Western with total protein normalization**, total lane volume is used to define the control.*

Step	Action
1	Select one of the following: • ***Membrane (target)*** image to adjust the target range definition • ***Membrane (control)*** image to adjust the control range definition 
2	In the ***Target Definition*** or ***Control Definition*** area, define start and end of the range with the arrows, or by typing, in the ***Range start (Rf)*** and ***Range end (Rf)*** fields.
3	Select which bands to use, most intense or all bands in range. If all bands are selected in the range, a sum of the volumes of all detected bands within the range will be used for normalization.

Relate values to lane

Step	Action
1	Check the box above the Bar chart, ***Relate values to lane***.
2	Select which lane to relate to. The bar chart will display values divided by the values in the lane that is related to. The lane that is related to will have value 1 in the bar chart.

Copy the Bar chart and paste into another application

Step	Action
1	Right-click in the graph and select ***Copy Graph***.
2	Paste the bar graph into another application, for example Microsoft Word.

Export Bar chart data to a table

Step	Action
1	Right-click in the graph and select ***Export.***
2	Browse for a folder, enter a name and click ***Save.*** The format is a text file (.txt)
3	• To open the the file in for example, Notepad, double-click the file. • To open the file in Microsoft Excel, select the file, right-click and select ***Open with.*** Choose Microsoft Excel.

Select what data to display in the Bar chart for different Experiment types

Select what data to show in the ***Bar chart*** by selecting the appropriate option in the ***Show*** drop-down list. One data type at a time can be displayed.

The tables below give a description of the available data types.

Western with endogeneous protein normalization

Select to show	Description
Normalized ratio	The normalized ratio (Volume $_{Target}$/Volume $_{Control}$) is displayed. The normalized ratio is available for the membrane overlay, membrane target, and membrane control image.
Target Volume	The volumes of the target bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000. The target volume is available for the membrane overlay, and the membrane target image.
Control Volume	The volumes of the control bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band (or bands, if using the option ***Use all bands in range***) from the original image, divided by 1000. The control volume is available for the membrane overlay, and membrane control image.

Western with total protein normalization

Select to show	Description
Normalized ratio	The normalized ratio (Volume $_{Target}$/Volume $_{Control}$) is displayed. The normalized ratio is available for the membrane overlay, membrane target, and membrane control image.
Target Volume	The volumes of the target bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000. The target volume is available for the membrane overlay, the membrane target, and the Cy5 gel image.

Select to show	Description
Control Volume	The volumes of the control bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000. The control volume is available for the membrane overlay, and membrane control image.

Easy Western

Select to show	Description
Ratio Cy3/Cy5 or ***Ratio Cy5/Cy3***	The ratio (Volume $_{\text{First primary antibody target}}$/ Volume $_{\text{Second primary antibody target}}$) is displayed. If Cy3 has been selected to detect the first primary antibody and Cy5 has been selected to detect the second primary antibody the ratio will be ***Ratio Cy3/Cy5***, or the opposite. This option is only available if two proteins are being targeted. The ratio is then available for the membrane overlay, membrane Cy5, and membrane Cy3 image.
Volume Cy5	The volumes of the Cy5 bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000. Volume Cy5 is available for the membrane overlay (if two proteins are being targeted), and the membrane Cy5 image.
Volume Cy3	The volumes of the Cy3 bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000. Volume Cy3 is available for the membrane overlay (if two proteins are being targeted), and the membrane Cy3 image.

Easy SDS-PAGE

Select to show	Description
Target Volume	The volumes of the target bands are displayed. The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000.

5.4.3 The Lane profile tab

Introduction

The lane profile tab is used to view the lane profile for one or several lanes in the gel or membrane images in any of the experiment types.

This tab is displayed as default for ***Easy-SDS PAGE*** experiments.

Lane profile graph

In the lane profile graph, the detected bands and the background signal for a selected lane in an image are viewed.

The lane profile graph shows the intensities as a function of pixel positions for one or all lanes. The intensity for a given profile position is determined as the average intensity for all pixels in the lane at that position.

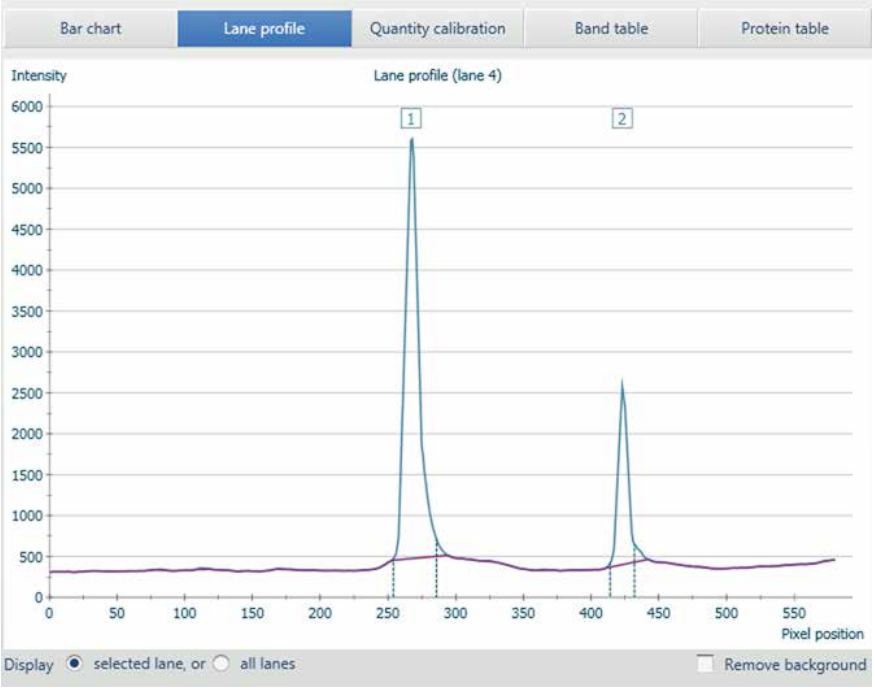
By default, the following is shown in the lane profile graph:

- the lane selected in the Image view
- intensities for pixel positions (peaks)
- the background signal (indicated with purple line)

A peak above the background signal with a band number displayed above it corresponds to a detected band.

The peak with band number 1 corresponds to the detected band with the highest molecular weight for that lane in the image.

When selecting a detected band in the lane profile graph by clicking on the band number (highlights the band number in blue) the corresponding band in the Image view is also selected.



Tip: *It may sometimes be easier to find bands that have not been detected in the automatic band detection by looking in the Lane profile graph rather than by looking in the Image view.*

Display options for the lane profile graph

The table below lists the different display options for the lane profile graph. The options can be found below the graph.

Option	Description
Display	• ***selected lane*** Select this radio button to show the selected lane in the Image view in the Lane profile graph. • ***all lanes*** Select this radio button to show all enabled lanes in the Lane profile graph simultaneously.

Option	Description
Remove background	The background signal is shown by default in the Lane profile graph. Check this box if you want to hide everything below the background signal in the Lane profile graph.

What can be found when looking at the Lane profile?

Usually the Lane profile will show all your bands in the image for that lane. Detected bands have a band number above the peak.

However, by looking at the lane profile, you may find for example:

- bands that have not been detected in the automatic band detection (peaks above the background signal that has no band number),
- that the background signal algorithm parameter (rolling ball circle) needs to be adjusted in order to get the volumes correctly calculated.

If you need to edit the detection of bands or lanes, click the ***Edit*** button and select the ***Detect bands*** tab or ***Detect lanes*** tab. See *Section 5.5 Edit analysis settings, on page 291* for information about how to edit lane or band detection.

5.4.4 The Band table tab

Introduction

The Band table tab is used to show selected data for the detected bands in a table format.

By default, the ***Lane number***, ***Sample Id***, ***Band number***, ***Volume***, ***MW***, ***Calibrated amount***, ***Band %***, and ***Rf*** values are shown.

Lane number	Sample Id	Band number	Volume	MW	Calibrated amount	Band %	Rf
2							
3	1	1	1393	40	N/A	93,2	0,467
3	1	2	101,5	36,1	N/A	6,79	0,494
4	1	1	1493	40,5	N/A	93,6	0,463
4	1	2	102,3	36,6	N/A	6,41	0,49
5	2	1	1401	40,3	N/A	90,8	0,465
5	2	2	142,5	36,4	N/A	9,23	0,492
6	2	1	1316	40,1	N/A	90,1	0,467
6	2	2	145,4	36,2	N/A	9,94	0,494
7	3	1	1207	40,3	N/A	86,7	0,465
7	3	2	184,6	36,2	N/A	13,3	0,494

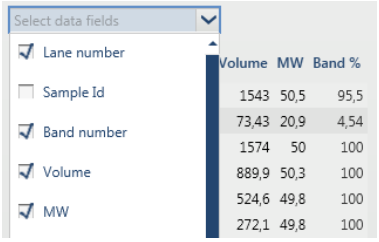
Purity check of a sample

By looking at the **Band%** data for a detected band (corresponding to the protein to be purified) within a lane, the purity of a sample containing that protein can be estimated.

Fraction analysis

By looking at the ***Volume*** or ***Band%*** data for a detected band in different lanes, the concentration or purity of a sample between fractions can be followed.

Select what data to display in the Band table

Step	Action
1	Click the ***Select data fields*** drop-down arrow. 
2	Select the data to be displayed in the table by checking/clearing the appropriate boxes. See *Display options for the Band table* below for descriptions of the options. ***Tip:*** *To reset the Band table to show the default data fields, check the* ***Default parameters*** *box at the end of the list.*
3	Click the ***Select data fields*** drop-down arrow to close the drop-down box.

Display options for the Band table

Data	Description
Lane number	Shows the lane number in which the detected band(s) is located. Only lanes that contain at least one detected band are displayed. ***Tip:*** *Lanes are expanded by default in the Band table, showing all band data for all lanes. By clicking the triangle in front of a lane number, that lane is collapsed.*

Data	Description
Sample Id	Shows the Sample Id for the lane if it was entered in the Sample layout table in the ***EXPERIMENT & SAMPLES*** workflow step. ***Tip:*** *You can enter and edit a Sample Id in the **EXPERIMENT & SAMPLES** workflow step at any time.*
Band number	The number of the detected band within each lane. Number 1 has the highest molecular weight.
Volume	The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000.
MW	Calculated molecular weight using the MW calibration curves ($M_r \times 10^3$)
Calibrated amount	Calculated quantity using the Calibration curve.
Band%	A measure of the band's volume divided by the sum of the volumes of all the bands in the lane.
Rf	Rf (Retardation Factor) is a measurement of position along the lane, relative to its length. By definition, the first position in each lane has an Rf of 0 and the last has an Rf of 1. There is a linear increase in Rf from start to finish.
Pixel position	The vertical y-position of the center of the band, measured in pixels.
Volume + background	The band volume before the background intensity has been removed, divided by 1000.
Peak height	The maximum value of the lane profile of the band, not including the background.
Peak + background	The maximum value of the lane profile of the band with the background value added.
Default parameter	Resets the Band table to show the default data fields.

Copy the Band table and paste into another application

Step	Action
1	Right-click in the table and select ***Copy table***. ***Note:*** ***Copy*** *will copy only the selected columns.* ***Export*** *will export all the columns, see instruction below.*
2	Paste the table into e.g., a Microsoft Excel sheet.

Export the Band table as a text file

Step	Action
1	Right-click in the table and select ***Export***.
2	Browse for a folder, enter a name and click ***Save***. The format is a text file (.txt)
3	• To open the the file in e.g., Notepad, double-click the file. • To open the file in Microsoft Excel, select the file, right-click and select ***Open with.*** Choose Microsoft Excel. ***Note:*** *All available data in the table is exported, not just the columns currently displayed. Included in the table are also experiment information and the settings used.*

5.4.5 The Quantity calibration tab

Introduction

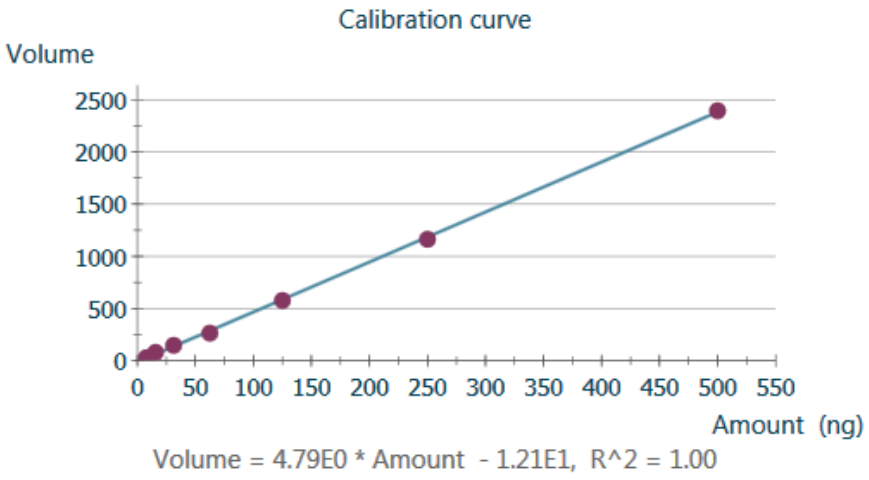
The quantity calibration tab is used to:

- view the automatically calculated calibration curve,
- adjust the Rf ranges for the quantity calibrants to be used in the calculation of the calibration curve.

This tab is displayed as default for ***Easy-SDS PAGE*** or ***Easy Western*** experiments containing quantity calibrants. The quantity calibrants must have been entered in the Sample table in the ***EXPERIMENT & SAMPLES*** workflow step.

Calibration curve

The calibration curve shows the amount of material as a function of the volume of a defined protein band, or a group of bands. The curve is calculated using linear regression. The equation of the curve and obtained coefficient of regression are displayed below the curve.



The calibration curve is used to calculate the amount of material in all bands in the image from the band volume (total sum of pixel intensities), based on the quantity calibrants entered in the ***EXPERIMENT & SAMPLES*** workflow step. The calibrated amounts are found in the ***Protein table*** and ***Band table***.

Annotations in the Image view

The detected bands in the lanes that were used in the calculation of the calibration curve are defined by the quantity calibrants range, marked by the two dotted lines in the Image view. The bands used in the calculation are annotated with a purple or a brown (added/edited bands) square.

Detected bands that are not included in the calculation of the curve are annotated with a white square.

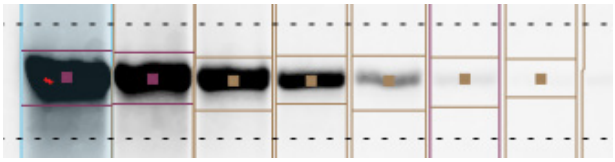


Image display

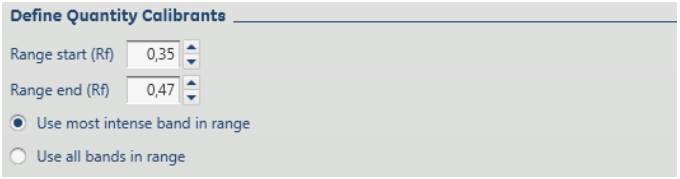
In ***Easy SDS-PAGE*** experiments, the quantity calibrants are visualized in the Gel Cy5 image. The quantity calibrants should have been individually pre-labeled with Cy5. Samples should have been pre-labeled using the same protocol and conditions.

In ***Easy Western*** experiments, the quantity calibrants and sample proteins are visualized in the membrane image due to the use of a specific primary antibody and labeled secondary antibody.

Define Quantity calibrants

If you have several detected bands in lanes containing your quantity calibrants and only one, or a group of bands in the lanes that should be used as quantity calibrants in the calibration curve calculation, adjust the ranges that define your calibrants

In the ***Define Quantity calibrants*** area below the graph:

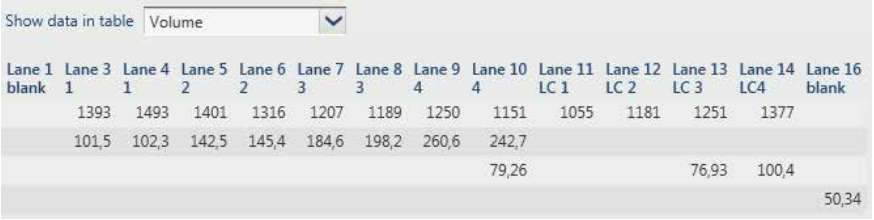
Step	Action
1	Define start and end of the range with the arrows, or by typing, in the ***Range start (Rf)*** and ***Range end (Rf)*** fields. • click the ***Range start (Rf)*** arrows, or type in a value, to move the dotted line defining the start of the quantity calibrants area in the Image view to the appropriate position • click the ***Range end (Rf)*** arrows, or type in a value, to move the dotted line defining the end of the quantity calibrants area in the Image view to the appropriate position 
2	Select which bands to use, most intense or all bands in range. If all bands are selected in the range, a sum of the volumes of all detected bands within the range will be used.
3	To delete a point from the calibration curve, go to ***EXPERIMENT & SAMPLES*** step and set the lane as Blank.
4	To restore default values, click the ***Default*** button.

5.4.6 The Protein table tab

Introduction

The Protein table tab shows data for detected bands in selected Membrane or Gel views.

Matched bands are in the same row position.



Purity check of a sample

By looking at the **Band%** data for a detected band (corresponding to the protein to be purified) within a lane, the purity of a sample containing that protein can be estimated.

Fraction analysis

By looking at the ***Volume*** or ***Band%*** data for a detected band in different lanes, the concentration or purity of a sample between fractions can be followed.

Display options for the Protein table

Select what data to show in the protein table by selecting the appropriate option in the ***Show data in table*** drop-down list. One data type at a time can be displayed.

The table below gives a description of the available data types.

Data	Description
Volume	The volume of a band is the sum of the intensities of all the pixels within the band from the original image, divided by 1000.
MW	Calculated molecular weight using the MW calibration curves ($M_r \times 10^3$)
Calibrated amount	Calculated quantity using the Calibration curve.

Data	Description
Band%	A measure of the band's Volume divided by the Total Volume of all the bands in the lane.
Rf	Rf (Retardation Factor) is a measurement of position along the lane, relative to its length. By definition, the first position in each lane has an Rf of 0 and the last has an Rf of 1. There is a linear increase in Rf from start to finish.
Pixel position	The vertical y-position of a band measured in pixels.
Volume + background	The band volume before the background intensity has been removed, divided by 1000.
Peak height	The maximum value of the lane profile of the band, not including the background.
Peak + background	The maximum value of the lane profile of the band with the background value added.

Copy the Protein table and paste into another application

Step	Action
1	Right-click in the table and select ***Copy table***. ***Note:*** ***Copy*** *will copy only the selected columns.* ***Export:*** *Export only includes the currently selected parameter , see instruction below.*
2	Paste the table into e.g., a Microsoft Excel sheet.

Export the Protein table as a text file

Step	Action
1	Right-click in the table and select ***Export***.
2	Browse for a folder, enter a name and click ***Save***. The format is a text file (.txt)

Step	Action
3	• To open the the file in e.g., Notepad, double-click the file. • To open the file in Microsoft Excel, select the file, right-click and select ***Open with.*** Choose Microsoft Excel. ***Note:*** *Included in the table when exported are also experiment information and the settings used.*

5.5 Edit analysis settings

Introduction

This section describes how to edit the analysis settings used in the evaluation of the results.

The following may be edited:

- lane detection
- band detection
- band matching
- molecular weight calibration

In this section

This section contains the following subsections:

5.5.1 Edit lane detection

Introduction

The automatic lane detection calculates and displays the lanes to be fitted on the gel and membranes. If the lane detection is not correct, or if the algorithm was unable to detect the lanes, a standard lane grid is shown as a starting point when looking at the images in the Image view.

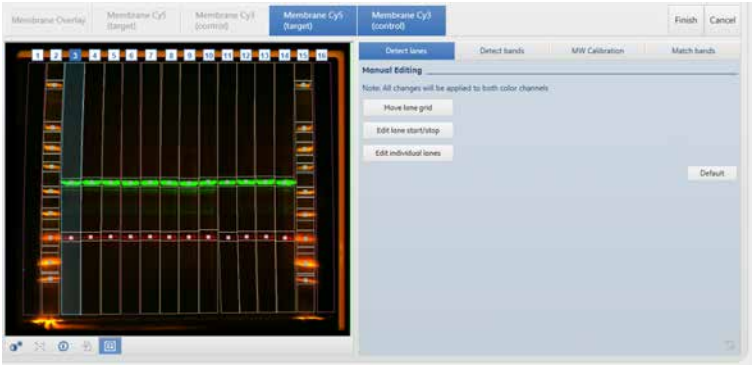
The lane detection is common for both membrane images, regardless of which image is displayed. The gel image has a separate lane detection.

The automatic lane detection can be edited by:

- moving the lane grid
- editing the lane start/stop
- editing the lane width and bending
- excluding lanes from being used in the calculations

Enter Edit mode for lane detection

Step	Action
1	In the ***EVALUATION*** workflow step, click the ***Edit*** button. *Result:* The ***Detect lanes*** tab is displayed.



Note:

When in Edit mode, an extra set of the Membrane images and the gel image tabs are displayed to the right of the ordinary tabs (which are dimmed).

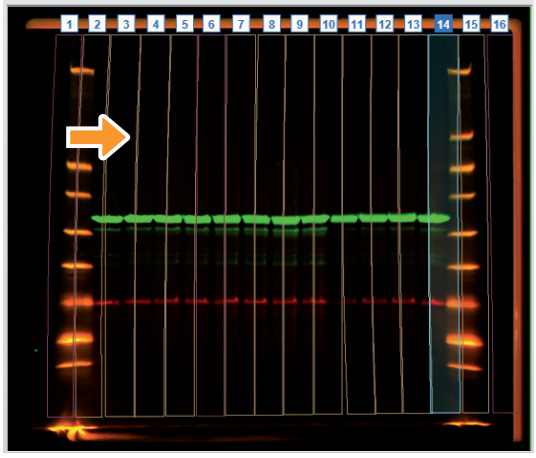
The tabs to the right are always used in Edit mode. The performed editing applies to the tab(s) highlighted blue in Edit mode.

Step	Action
2	Select the image you want to edit and then continue with the editing as described below.

Move lane grid

The complete lane grid can be moved to fit the actual position of the bands of the separated sample.

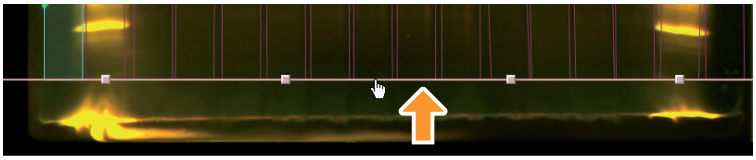
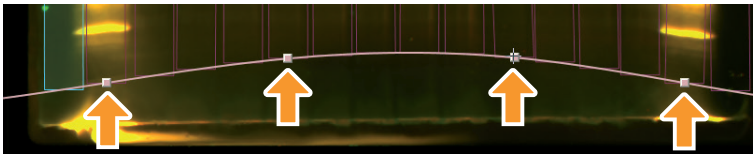
Step	Action
1	Click ***Move lane grid***.
2	Click in the grid and move.



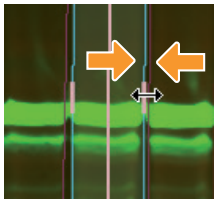
Edit lane start/stop

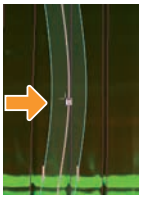
The lane start and stop might need to be edited, for example if the front is uneven, to improve the band Rf values.

Step	Action
1	Click ***Edit lane start/stop***.

Step	Action
2	Use the purple horizontal lines to set the desired start and stop of the lanes. 
3	Use the rectangular handles on the purple horizontal lines to bend the start and stop of the lanes. 

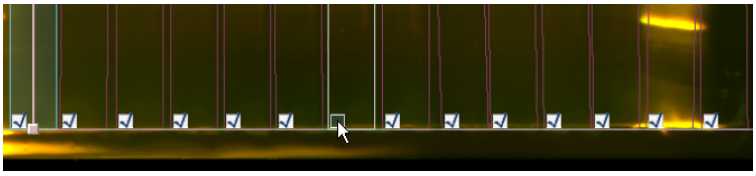
Edit lane width and bending

Step	Action
1	Click ***Edit individual lanes***.
2	To re-size the individual lane width: • select the lane • move the mouse over one of the two rectangular handles (the mouse pointer changes to a double pointed arrow) • drag the handle to re-size the lane width 

Step	Action
3	There are four handles that can be used in combination to bend/move the lane to the appropriate position. To move or edit the shape of a lane: • Select the lane. • Move the mouse over one of the square handles to adjust the bending. • If appropriate, repeat the steps above for another handle. 
4	If appropriate, continue to edit the next lane.

Exclude a lane from calculations

Individual lanes can be excluded from being used in calculations, if for example you want to compare only a subset of your samples.

Step	Action
1	Click ***Edit individual lanes***.
2	Clear the check box at the lower end of the lane to exclude a lane from the calculation. 

Restore default settings

Step	Action
1	Click ***Default*** to restore default lane detection. ***Note:*** *When restoring lane detection to default, the experiment is re-evaluated with the current band parameters, that is, the band detection, matching and molecular weight are also re-calculated. Therefore, this can be used to get back to the original evaluation, if you for example regret the performed editing of results. However, default values for Rolling Circle, Sensitivity, Match tolerance etc are not achieved.*
2	In the displayed dialog, click ***OK.***

Undo an editing operation

To undo an editing operation, click  in the lower-right corner of the screen.

Note: *Manual changes of lane, band and matches are restored. However, changes of parameters do not restore default values.*

Finish editing

- To exit the Edit mode and apply any changes made, click the ***Finish*** button.
- To edit settings on another tab, click that tab and edit as appropriate. When clicking ***Finish***, all changes on all tabs will be applied.
- To exit the Edit mode without applying any changes made, click the ***Cancel*** button.

Note: *If you change your mind about result editing, it is possible to perform a new complete evaluation of the image data for an experiment. In Edit mode, select the **Detect lanes** tab and click the **Default** button. However, default values of parameters, e.g., Rolling circle, Sensitivity and Match tolerance, are not restored.*

Note: *Bands in the molecular weight markers are not affected by the detect band setting. To edit MW bands go to **MW Calibration**.*

5.5.2 Edit band detection

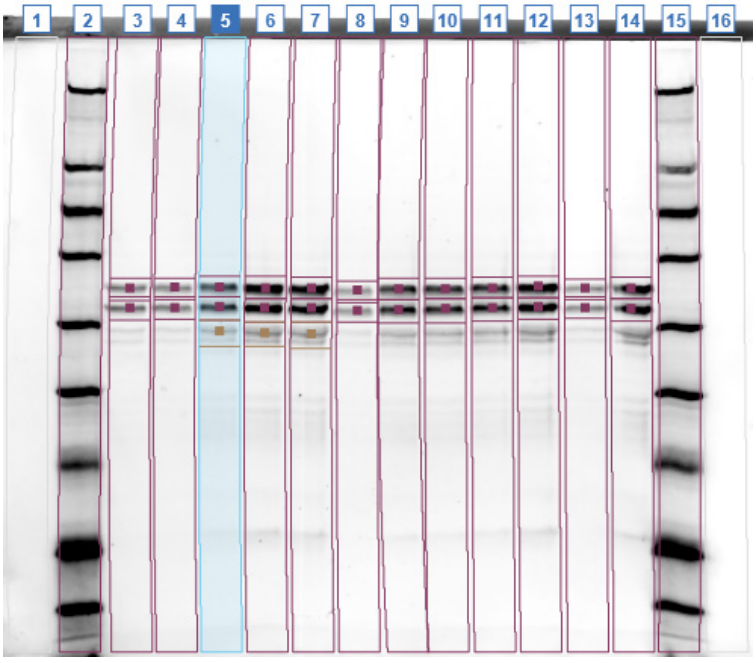
Introduction

The band detection settings can be edited by:

- changing the parameters for automatic band detection
- manually adding or deleting bands
- changing the band width

Enter Edit mode for band detection

Step	Action
1	In the ***EVALUATION*** workflow step, click the ***Edit*** button. *Result:* The ***Detect lanes*** tab is displayed. ***Note:*** *When in Edit mode, an extra set of the Membrane images and the gel image tabs are displayed to the right of the ordinary tabs (which are dimmed).* *The tabs to the right are always used in Edit mode. The performed editing applies to the tab(s) highlighted in blue in Edit mode.*

Step	Action
2	To edit the band detection settings or manually add/delete bands, click the ***Detect bands*** tab. *Result*: The view for band detection is displayed. 
3	To edit the band detection, continue with the instructions below. ***Note:*** *Band detection in MW marker lanes can not be adjusted the in the **Detect bands** tab. The MW marker detection is separate and can only be edited in the **MW Calibration** tab.*

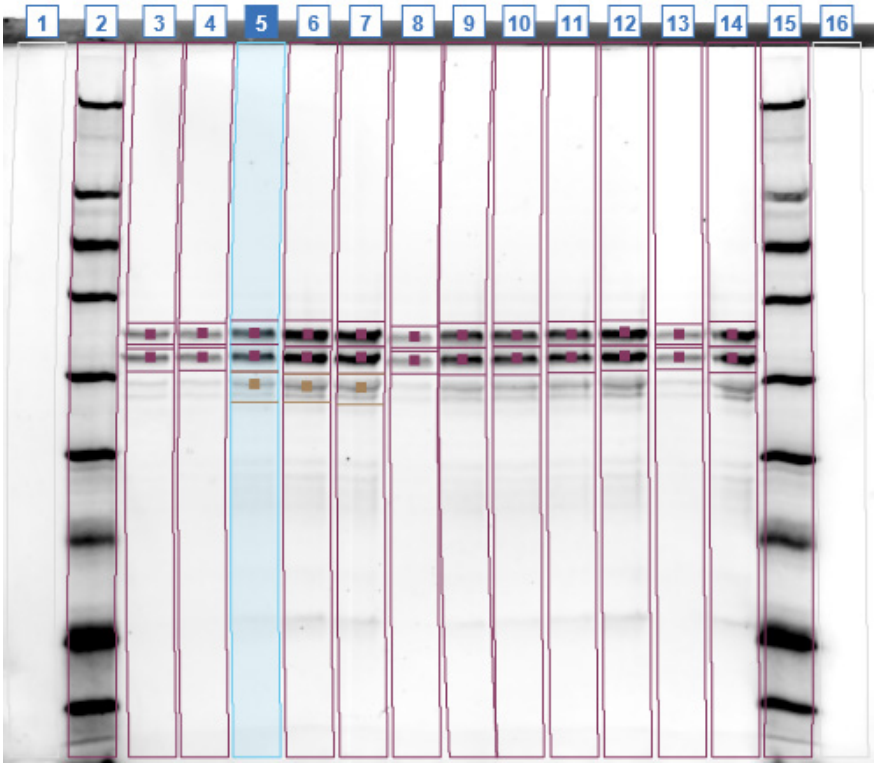
Overview - band detection display

Image view

The Image view shows the selected Membrane/Gel image with bands.

When showing annotations, detected bands are indicated by a square and a line above (band start) and a band below (band stop) the black Cy3 or Cy5 signal (depending on the image being viewed).

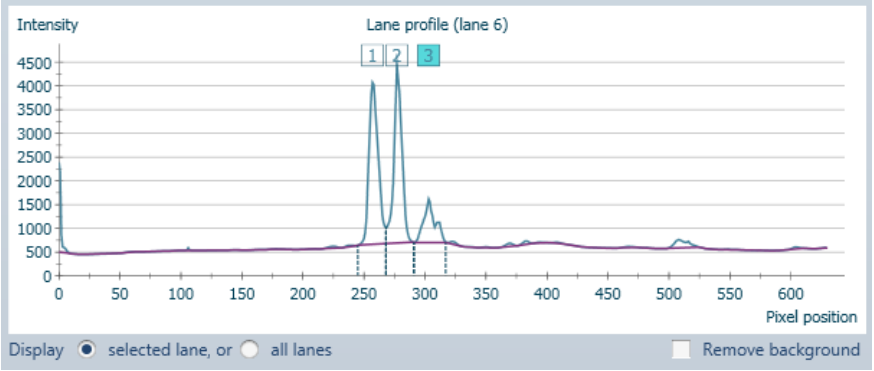
- Purple square and lines represent a band
- Yellow square and lines represent any selected band (the corresponding band number box is highlighted in blue in the lane profile)
- Brown square and lines represent an edited band



Lane profile

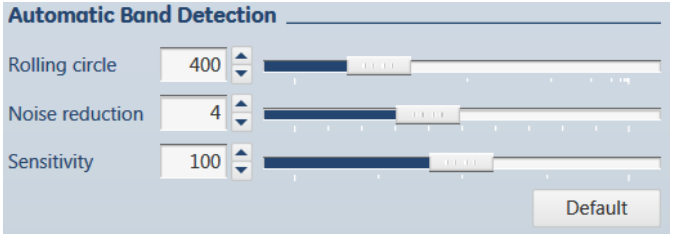
The lane profile of a selected lane is displayed to the right. Detected bands have numbered boxes above each peak.

Select a lane in the Image view to display the lane profile for that lane.



For more information about Lane profiles, see *Section 5.4.3 The Lane profile tab, on page 278*

Adjust Automatic band detection

Step	Action
1	To adjust the Automatic band detection, change the settings of the parameters in the ***Automatic Band Detection*** area. ***Note:*** *For pre-labeled control in total protein normalization, only background subtraction can be adjusted using the Rolling circle parameter.* 
2	To restore default settings, click ***Default***.

Band detection parameters

The table below describes the parameters that can be edited for automatic band detection.

Parameter	Description.
Rolling circle	The Rolling circle method requires you to enter a value for the size of the rolling circle. This method calculates the background as if a disc, with the radius you have entered, were rolling underneath the lane profile. The larger the radius of the disc, the less the background rises with the profile.
Noise reduction	This parameter represents the degree to which small local peaks should be ignored on the profile and is designed to eliminate noise in the image. Noise reduction has no effect on the profile itself, only the number of peaks detected. In general, the higher the Noise Reduction value, the fewer peaks detected.
Sensitivity	This parameter represents how pronounced the band must be from its surrounding area in the lane. Increase the value to increase the sensitivity of the band detection.

Note: *None of the parameters above will affect the MW calibration. Edit the calibration in the **MW Calibration** tab.*

Note: *For pre-labeled control in total protein normalization, only the Rolling circle background subtraction parameter is applicable.*

Manually add or delete bands

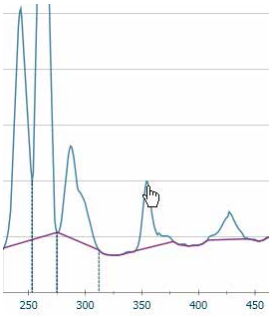
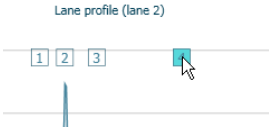
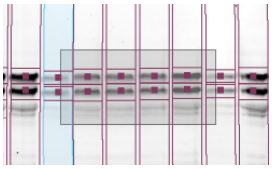
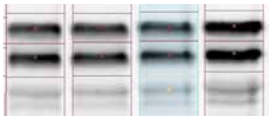
Bands can be added/deleted in the Image view or in the Lane profile view.

Note: *Bands in the Cy5 Control image in Total protein normalization experiments can not be edited.*

To start editing of bands:

Step	Action
1	Click in the image view to select a lane.
2	Click the ***Edit bands*** button located below the Lane profile in the ***Manual Editing*** area. 

The table below describes how to add/delete bands using the Lane profile and Image view:

View	Action
Lane profile view	• To add a band, place the cursor at the top of a peak and **left-click**.  • To delete a band, place the cursor on the peak number box and **right-click**. 
Image view	• To add or delete a band, zoom in on the area of interest by holding down the left-mouse-button and create a box.  • To add a band, place the cursor on an undetected band in the image and **left-click**. The band will be annotated with a brown square indicating that it has been added manually.  • To delete a band, place the cursor on the band and **right-click**.

Tip: *It may sometimes be easier to find bands that have not been detected in the automatic band detection by looking in the Lane profile graph rather than by looking in the Image view.*

Edit band start/stop

Step	Action
1	Click ***Edit bands***.
2	Click and drag the horizontal lines to set the desired start and stop of the band. 

Undo an editing operation

To undo an editing operation, click  in the lower-right corner of the screen.

Note: *Manual changes of lane, band and matches are restored. However, changes of parameters do not restore default values.*

Finish editing

- To exit the Edit mode and apply any changes made, click the ***Finish*** button.
- To edit settings on another tab, click that tab and edit as appropriate. When clicking ***Finish***, all changes on all tabs will be applied.
- To exit the Edit mode without applying any changes made, click the ***Cancel*** button.

Note: *If you change your mind about result editing, it is possible to perform a new complete evaluation of the image data for an experiment. In Edit mode, select the **Detect lanes** tab and click the **Default** button. However, default values of parameters, e.g., Rolling circle, Sensitivity and Match tolerance, are not restored.*

Note: *Bands in the molecular weight markers are not affected by the detect band setting. To edit MW bands go to **MW Calibration**.*

5.5.3 Edit band matching

Introduction

The band matching shows which bands are matched as the corresponding bands in the different lanes. Bands are matched if they lie within the area defined by set the match tolerance value (± Rf-value).

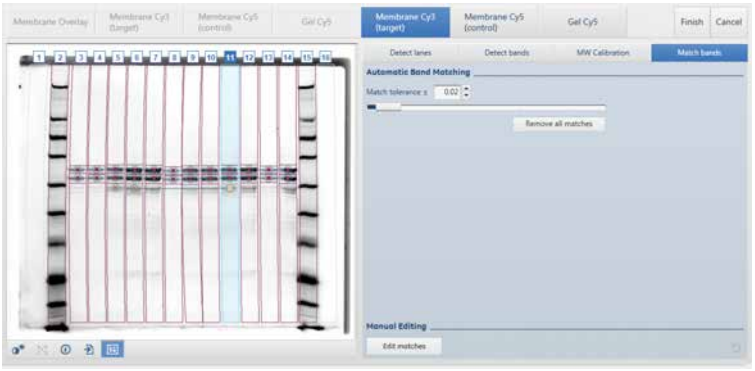
If there are several bands in a lane that can be matched to another band in a nearby lane (i.e., several bands lie within the defined match tolerance area), the band that has the most similar Rf value as the band in the nearby lane will be matched.

Note: *Bands in the molecular weight markers are not included in matching.*

The band matching is used in the result display in the ***Protein table*** tab. Matched bands are placed in the same row in the table for direct comparison between lanes.

Enter Edit mode for band matching

Step	Action
1	In the ***EVALUATION*** workflow step, click the ***Edit*** button. *Result:* The ***Detect lanes*** tab is displayed. ***Note:*** *When in Edit mode, an extra set of the membrane images and the gel image tabs are displayed to the right of the ordinary tabs (which are dimmed).* *The tabs to the right are always used in Edit mode. The performed editing applies to the tab(s) highlighted in blue in Edit mode.*

Step	Action
2	To edit the band matching settings or manually add/delete bands to/from band matching, click the ***Match bands*** tab. *Result:* The settings for band matching are displayed on the ***Match bands*** tab. The Image view shows the matched bands. 
3	To edit the band matching, continue with the instructions below.

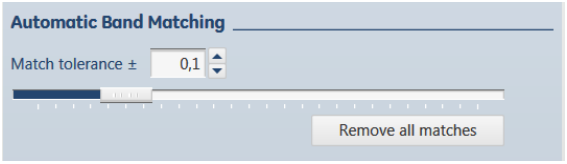
Overview - band matching display

The Image view shows the selected Membrane/Gel image with bands.

The following band annotation is used:

- detected bands are annotated with purple squares
- edited bands are annotated with brown squares
- matched bands are annotated with blue circles connected with lines
- a selected band in the Image view is annotated with a yellow square

Adjust Automatic band matching

Step	Action
1	Adjust the matching by changing the ***Match tolerance±*** value. 
2	To remove all matches, click ***Remove all matches***. ***Tip:*** *The **Remove all matches** function is useful when you only have one protein of interest in a sample where several proteins are present.*

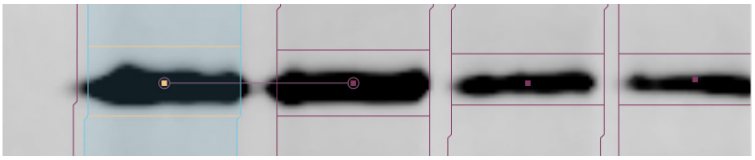
Manual editing of matching

Band matches can be manually created, added to an existing match or removed from an existing match. To start manual editing of a match:

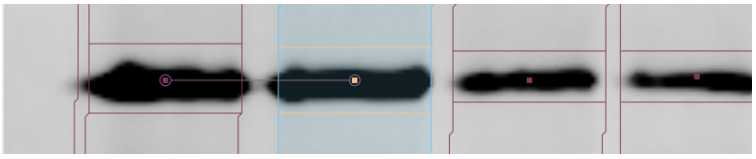
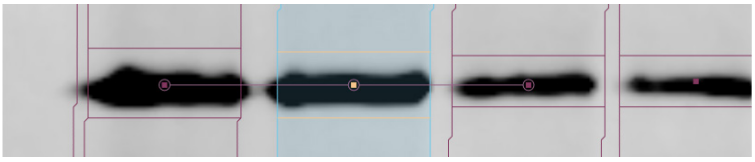
Step	Action
1	Zoom-in on the area of interest in the image (left-click and draw a box).
2	Proceed with the appropriate instruction below for editing of matches.

Create a match between two unmatched bands

Step	Action
1	Click ***Edit matches***.
2	Left-click in the lane with the first band.
3	Left-click on the first band. *Result:* 

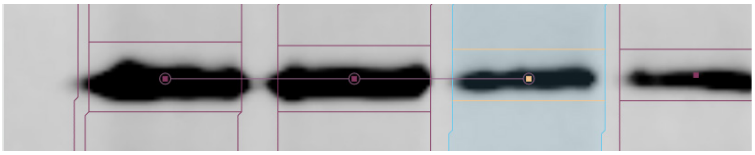
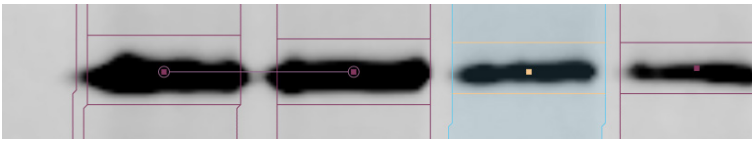
Step	Action
4	Right-click on the second band, which will be added to the match. *Result:* 

Add a new band to an existing match

Step	Action
1	Click ***Edit matches***.
2	Left-click in the lane with the last band of a match.
3	Left-click on the last band. *Result:* 
4	Right-click on the band, which will be added to the match. *Result:* 

Remove a band from a match

Step	Action
1	Click ***Edit matches***.

Step	Action
2	Left-click on one of the bands in the match. *Result:* 
3	Right-click on the band you want to remove from the match. *Result:*  Several bands can be removed from a match by right-click on each of them.

Undo an editing operation

To undo an editing operation, click  in the lower-right corner of the screen.

Note: *Manual changes of lane, band and matches are restored. However, changes of parameters do not restore default values.*

Finish editing

- To exit the Edit mode and apply any changes made, click the ***Finish*** button.
- To edit settings on another tab, click that tab and edit as appropriate. When clicking ***Finish***, all changes on all tabs will be applied.
- To exit the Edit mode without applying any changes made, click the ***Cancel*** button.

Note: *If you change your mind about result editing, it is possible to perform a new complete evaluation of the image data for an experiment. In Edit mode, select the **Detect lanes** tab and click the **Default** button. However, default values of parameters, e.g., Rolling circle, Sensitivity and Match tolerance, are not restored.*

Note: *Bands in the molecular weight markers are not affected by the detect band setting. To edit MW bands go to **MW Calibration**.*

5.5.4 Edit molecular weight calibration

Introduction

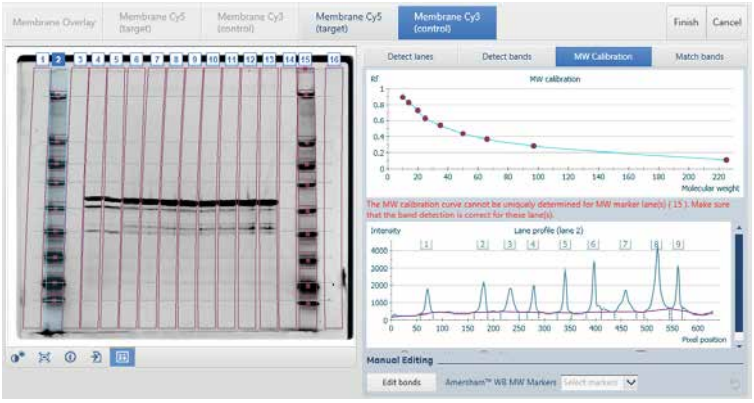
The molecular weight calibration curve is calculated based on the lanes set to ***Amersham™ WB MW markers*** in the ***EXPERIMENT & SAMPLES*** workflow step.

The molecular weight calibration can be edited by:

- manually adding/deleting bands in the marker lanes
- excluding individual marker bands from the calculation
- changing the width of marker bands

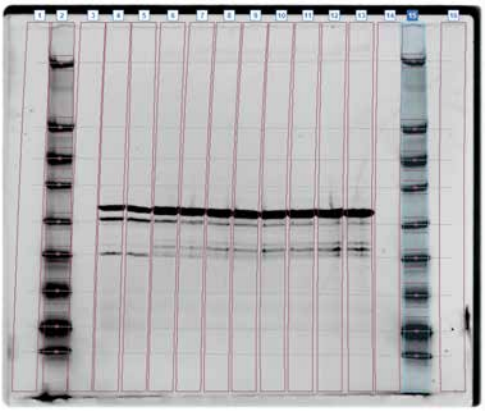
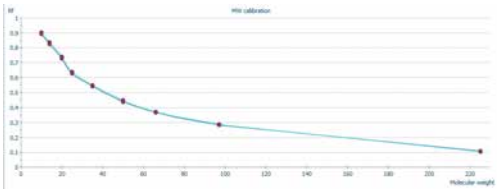
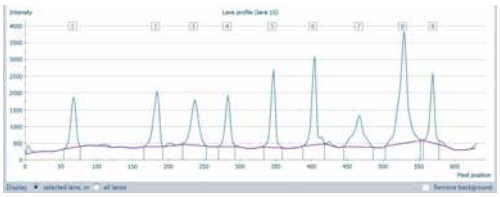
Enter Edit mode for molecular weight calibration

Step	Action
1	In the ***EVALUATION*** workflow step, click the ***Edit*** button. *Result:* The ***Detect lanes*** tab is displayed. ***Note:*** *When in Edit mode, an extra set of the membrane images and the gel image tabs are displayed to the right of the ordinary tabs (which are dimmed).* *The tabs to the right are always used in Edit mode. The performed editing applies to the tab(s) highlighted in blue in Edit mode.*

Step	Action
2	To edit the molecular weight calibration, click the ***MW calibration*** tab. *Result:* The following is displayed: • Image view Membrane/Gel image with annotated detected MW marker bands. • ***MW calibration*** curves graph • ***Lane profile*** for a selected lane • If MW calibration has failed, an error message is shown between the MW calibration curve and the Lane profile. 
3	To edit the molecular weight calibration, continue with the instructions below.

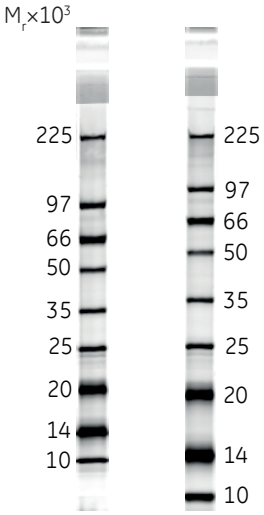
Display for molecular weight calibration

The following is displayed for molecular weight calibration:

Display	Description
Image view 	Membrane/Gel image with annotated detected MW marker bands. If multiple MW markers are present, green lines are drawn between each corresponding molecular weight marker band. MW marker bands can be edited in this view.
MW calibration curves graph 	Shows the molecular weight calibration curves, one for each marker lane. To display the curve for a specific lane, click the lane in the Image view. ***Note:*** *The different curves and points usually overlaps. The curve for the right most lane is displayed on top of the other curves.* If MW calibration has failed, a message is displayed that provides an explanation.
Lane profile for a selected lane 	Shows the lane profile for a selected lane, or for all MW marker lanes, in the Image view. MW marker bands can be edited in this graph.

Check the MW calibration

Step	Action
1	Make sure that the MW marker bands in the marker lanes have been detected correctly. The images below show the correct display of the nine MW marker bands for gradient (left) and homogeneous (right) gels:



- If a MW marker band has not been detected, but is visible in the image view, add it as a detected MW calibration band.
- If for any reason a band has been detected in the MW marker lane that is not a MW marker band, delete the band.

See *Edit bands in the MW marker lanes, on page 313* for more information.

Tip:

If multiple MW markers are present, green lines are drawn between each corresponding molecular weight marker band for the marker lanes in the Image view. If the lines are not horizontal, this indicates that a band may be missing in one of the molecular weight marker lanes or an extra band may have been detected.

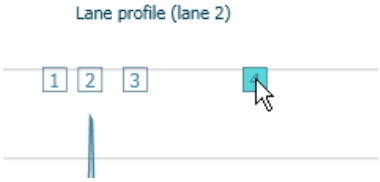
Tip:

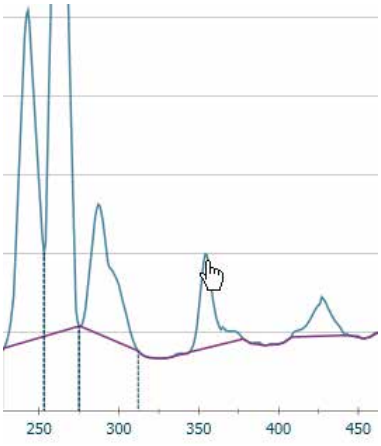
An error message is displayed below the molecular weight calibration curve if the software detects any errors in the MW calibration.

Step	Action
2	If there is a MW marker band missing from all marker lanes, also after editing the bands in the marker lanes (for example if the electrophoresis has run too short or too long or if the marker is not well separated), check that this band(s) has been excluded in the ***Select markers*** drop-down list. If not, exclude the band from being used in the algorithm, by deselecting it in the ***Select markers*** drop-down list. See *View/edit MW markers in Select markers drop-down list, on page 314* for information about how to exclude MW marker bands.

Edit bands in the MW marker lanes

Step	Action
1	Click the ***Edit bands*** button located in the ***Manual Editing*** area below the Lane profile.
2	Delete in the Lane profile detected bands which are not MW markers. Place the cursor on the peak number box and **right-click**.



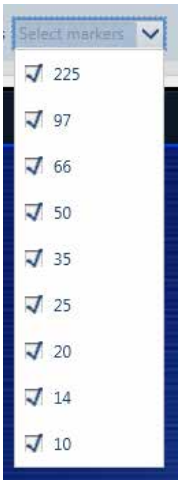
Step	Action
3	Add detections (left-click) of bands which are MW markers. Place the cursor at the top of a peak and **left-click**. 
4	Check that lines between markers are horizontal. These are used for MW calibration for all sample and quantity calibrant lanes. For more information about the molecular weight calibration, see below.

Note: *If only one lane is used for MW calibration, all lanes will be MW calibrated using the curve of that lane.*

View/edit MW markers in Select markers drop-down list

Select in the ***Amersham WB™ MW Markers*** scroll list the markers to be included in the MW calibration curve.

It can be relevant to exclude a MW marker band(s), for example when the electrophoresis has run too short or too long. In those cases one of the markers may be missing in the visualized gel image.



Description of the molecular weight calibration algorithm

Calculated calibration curves are used to calculate the molecular weights. The table below briefly describes how molecular weights are calculated using calibration curves determined by interpolation from lanes run with MW markers.

Stage	Description
1	Straight green lines are drawn between each corresponding MW marker band.
2	The intersection of these lines with the other lanes gives new Rf values.
3	These Rf values are used together with the assigned MW values to generate a calibration curve for each lane.
4	The bands in each lane are assigned MW values using this calibration curve. ***Note:*** *The calculated calibration curves for the sample lanes are not included in the displayed MW Calibration curve view.*

Note: *If only one lane is used for MW calibration, all lanes will be MW calibrated using curves created from the rf values of that lane.*

Undo an editing operation

To undo an editing operation, click  in the lower-right corner of the screen.

Note: *Manual changes of lane, band and matches are restored. However, changes of parameters do not restore default values.*

Finish editing

- To exit the Edit mode and apply any changes made, click the ***Finish*** button.
- To edit settings on another tab, click that tab and edit as appropriate. When clicking ***Finish***, all changes on all tabs will be applied.
- To exit the Edit mode without applying any changes made, click the ***Cancel*** button.

Note: *If you change your mind about result editing, it is possible to perform a new complete evaluation of the image data for an experiment. In Edit mode, select the **Detect lanes** tab and click the **Default** button. However, default values of parameters, e.g., Rolling circle, Sensitivity and Match tolerance, are not restored.*

Note: *Bands in the molecular weight markers are not affected by the detect band setting. To edit MW bands go to **MW Calibration**.*

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6 Maintenance

About this chapter

This section lists the periodic maintenance activities that shall be performed by the user of the Amersham WB analyzer, as well as maintenance activities that should be performed when required.

Precautions

WARNING

Do not use Amersham WB analyzer if it is not working properly, or if it has suffered any damage, for example:

- damage to the power cord or its plug
- damage caused by dropping the equipment
- damage to other parts which can affect function

WARNING

Electrical shock hazard. All repairs should be done by service personnel authorized by GE. Do not open any covers or replace parts unless specifically stated in the user documentation.

WARNING

Disconnect power. Always disconnect power from the instrument unit before replacing any component on the instrument unit, unless stated otherwise in the user documentation.

WARNING

When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable personal protective equipment. For example NaOH is corrosive and therefore dangerous to health.

WARNING

Only spare parts and accessories that are approved or supplied by GE may be used for maintaining or servicing the system.

WARNING

When replacing a damaged power cord, use a replacement cord of the same type and dimensions and in conformance with all applicable local electrical code requirements and approved by GE.

WARNING

Always disconnect the mains supply before replacing the mains fuse. For continued protection against risk of fire, replace only with a fuse of the same type and rating as stated on the instrument unit labels.

CAUTION

When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

In this chapter

This chapter contains the following sections:

6.1 Maintenance program

Introduction

This section lists the maintenance activities that should be performed by the user of the Amersham WB analyzer. Maintenance is divided into:

- After each run
- Weekly maintenance
- Monthly maintenance
- Annual maintenance
- Maintenance when required

Periodic maintenance program

The following periodic maintenance should be performed by the user of the Amersham WB analyzer.

Interval	Maintenance action	Instruction
After each run in the Elpho & scan unit		
	• Wipe off any liquid on the card plate • Wipe off any liquid or dirt from the protective glass in the sealing lid • Inspect the glass of the sealing lid for scratches or marks and check that the glass is firmly attached to the frame • Inspect the sealing lid hatch when the frame is closed and remove any dirt	Use a lint-free cloth or paper that does not scratch the protective glass or the card plate. Use a lint-free cloth wetted in 50% ethanol to remove stains. Contact Service if you need to replace a part of the sealing lid.
	Clean the buffer strip holders	Clean the buffer strip holders using running water to remove salts and buffer. Leave the buffer strip holders to air dry upside down.
	Clean the membrane adapters	Clean the membrane adapters, using a lint-free cloth and place them in the storage space in the antibody compartment.

Interval	Maintenance action	Instruction
After each run in the Western unit		
	Clean the transfer flow path	If the transfer flow path was not cleaned after transfer was completed, run the cleaning procedure by clicking ***Control*:*Clean Transfer flow path*** in the menu bar.
	Clean the probing flow path	If the probing flow path was not cleaned after probing was completed, run the cleaning procedure by clicking ***Control*:*Clean Probing flow path*** in the menu bar.
	Clean the transfer holder	See *Clean the transfer holder, on page 207*.
	Clean the drying holders	Clean the drying holders, using a lint-free cloth, and place them in the drying compartment.
	Remove any particles in the transfer tank	Inspect the tank and remove any remains of gel or paper fibers using tweezers. Check the transfer tank filter and replace if dirty, see *Replace transfer tank filter, on page 336*.
	Clean the upper part of the transfer tank	Wipe the upper part of the transfer tank with a wet tissue.

Interval	Maintenance action	Instruction
Weekly (or when the instrument will not be used for a few days)		
	Maintenance clean of the transfer flow path	See *Section 6.2.1 Weekly cleaning of the transfer and probing flow paths, on page 325*.
	Maintenance clean of the probing flow path	See *Section 6.2.1 Weekly cleaning of the transfer and probing flow paths, on page 325*.
	Inspect, and replace if damaged, the: • pumps • tubing • air filter • inlet filter	See *Section 6.2.2 Replacement procedures, on page 328* for information about how to replace pump heads, air filter and inlet filter. Contact Service if you need to replace tubing.
	Inspect and tighten the tubing connectors	

Interval	Maintenance action	Instruction
Annually		
	Replace air filter in dryer module The air filter may need to be replaced more frequently if the external environment is more harsh than normal lab environments with respect to dust in the air.	See *Replace air filter, on page 329*.
	Replace transfer pump head	See *Replace probing or transfer pump head, on page 331*.
	Replace probing pump head	See *Replace probing or transfer pump head, on page 331*.

Interval	Maintenance action	Instruction
When required		
	Clean the drying compartment	Clean the drying compartment from any dust or particles using a lint-free cloth wetted with ultra pure water. Let the compartment dry before use.
	Replace transfer electrodes	See *Replace transfer electrodes, on page 334*.
	Replace buffer strip holders	See Amersham WB system User Manual for ordering information.
	Replace the mains fuse	See *Replace the mains fuse, on page 337*.
	Replace damaged power cord	See *Precautions* in the beginning of this chapter.
	Replace the filter in the transfer tank	Inspect the filter and if dirty or damaged, replace it. See *Replace transfer tank filter, on page 336*.
	Replace the inlet filters	See *Replace the inlet filters, on page 336*.

6.2 Maintenance instructions

Introduction

This section describes the maintenance to be performed on the Amersham WB analyzer.

In this section

This section contains the following subsections:

6.2.1 Weekly cleaning of the transfer and probing flow paths

Introduction

Perform maintenance cleaning of the transfer flow path and the probing flow path weekly, or if the unit will not be used for a couple of days.

Maintenance cleaning of transfer flow path

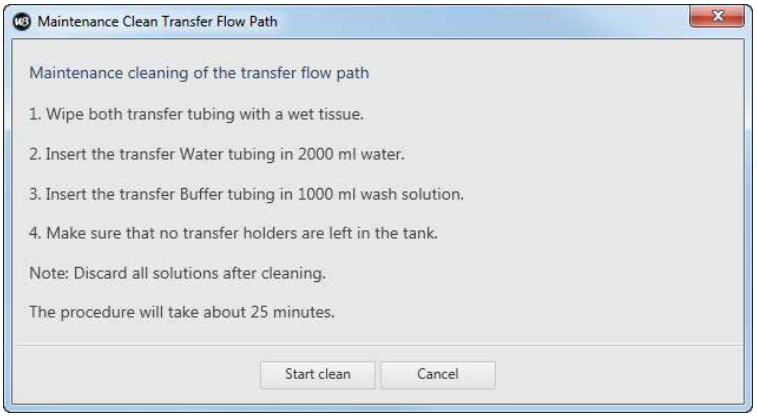
The procedure will take about 25 minutes. Probing and drying can be run in parallel.

Note: *Make sure there are no transfer holders left in the tank.*

WARNING

When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable personal protective equipment. For example NaOH is corrosive and therefore dangerous to health.

Step	Action
1	Select in software, ***Control:Maintenance clean Transfer flow path*** or click ***Maintenance clean Transfer*** button in the software start screen. *Result:* The ***Maintenance Clean Transfer Flow Path*** dialog opens.



2	• Wipe the upper part of the transfer tank and both the transfer tubing with a wet tissue. • Check that the transfer tank filter is intact and clean. If needed, replace the filter.

Step	Action
3	Immerse the **T Water** tubing in a bottle containing 2000 ml of ultra pure water.
4	Immerse the **T Buffer** tubing in a bottle containing 1000 ml 0.5 M NaOH.
5	Click the ***Start clean*** button in the dialog.
6	When cleaning is finished move the tubing into an empty bottle.

Note: *Discard all solutions after cleaning.*

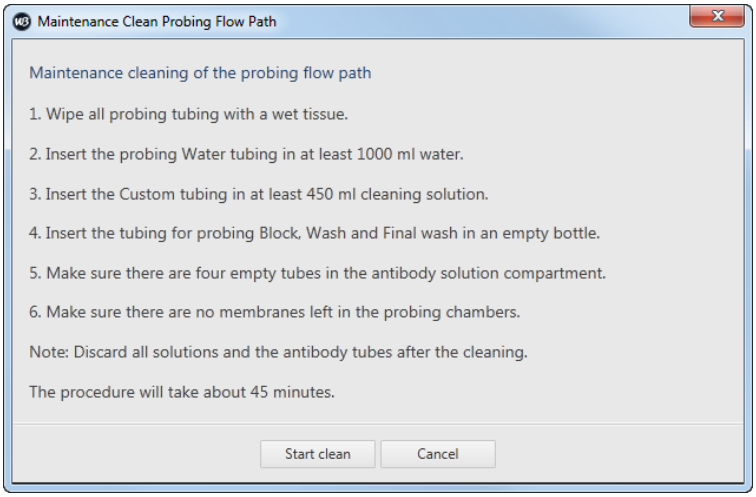
Maintenance cleaning of probing flow path

The procedure will take approximately 45 minutes. Transfer and drying can be run in parallel.

Note: *Make sure there are no PVDF cards left in the probing chambers.*

WARNING

When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable personal protective equipment. For example NaOH is corrosive and therefore dangerous to health.

Step	Action
1	Select in software, ***Control:Maintenance clean Probing flow path*** or click ***Maintenance clean Probing*** button in the software start screen. *Result:* The ***Maintenance Clean Probing Flow Path*** dialog opens. 
2	Wipe the probing tubing, and the lid and edges of the probing chamber with a wet tissue.
3	Immerse the **P Water** tubing in a bottle containing at least 1000 ml of ultra pure water.
4	Immerse the **P Custom** tubing in a bottle containing at least 450 ml 0.5 M NaOH.
5	Place **P Block**, **P Wash** and **P Final Wash** tubing in an empty waste bottle.
6	Insert and connect **four** clean empty 15 ml tubes in the antibody compartment.
7	Click ***Start clean*** in the dialog.
8	When cleaning is finished move the tubing into an empty bottle.

Note: *Discard all solutions and antibody tubes after cleaning.*

6.2.2 Replacement procedures

Introduction

This section describes how to replace the:

- air filter
- transfer and probing pump heads
- transfer electrodes
- transfer tank filter
- inlet filters
- mains fuse

Maintenance accessories

The following accessories are needed for maintenance of the Amersham WB analyzer:

- pump head (for transfer pump or probing pump)
- air filter
- transfer tank filter
- transfer electrodes
- inlet filter set
- fuse (for correct fuse. see *Section 8.1 System specifications, on page 353*)

Precautions

WARNING

Disconnect power. Always disconnect power from the instrument unit before replacing any component on the instrument unit, unless stated otherwise in the user documentation.

WARNING

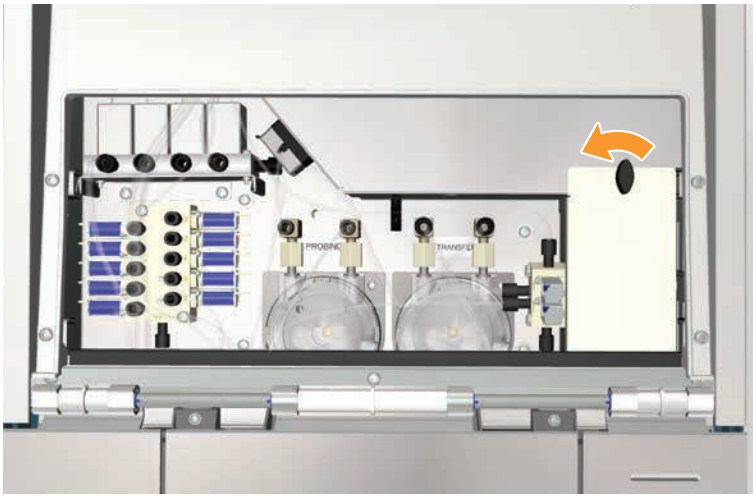
Remove any bottles from the bottle rack before opening the Service compartment lid.

Replace air filter

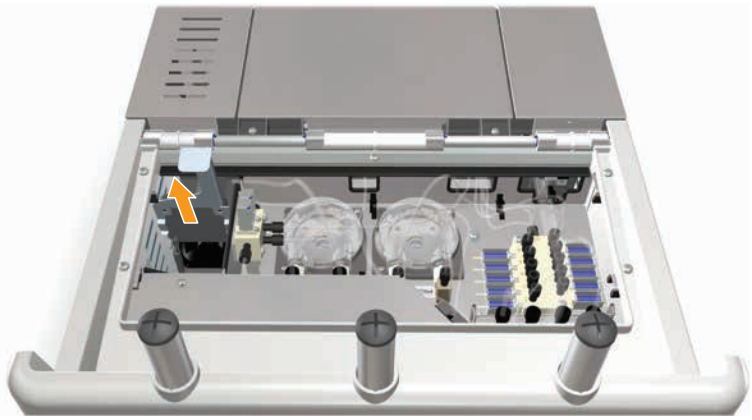
The instruction below describes how to replace the air filter.

Step	Action
1	Open the service compartment lid.

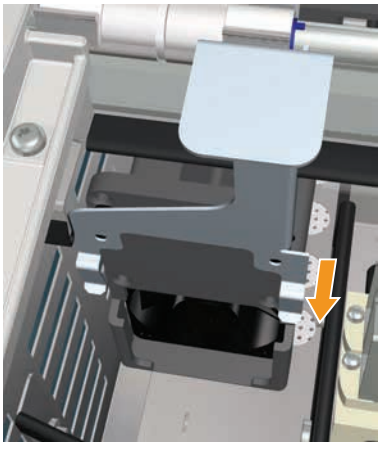
2	Open the lid above the compartment where the filter is located by turning the nut counter-clockwise and removing it.



Step	Action
3	Remove the filter from the frame by pulling it up using the attached holder.



4	Remove the top part of the plastic grid on the holder, where the filter is located.
5	Replace the filter with a new filter and fasten the top part of the plastic grid.
6	Insert the holder with a new filter into the guides in the frame.



7	Put the lid of the filter compartment back into place and fasten the nut by turning it clockwise.
8	Close the service compartment lid.

Replace probing or transfer pump head

The instruction below describes how to replace a pump head, using the probing pump as an example. Replacing the transfer head is similar.

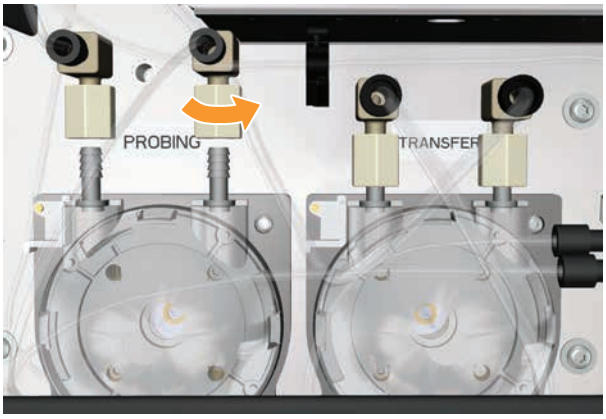
Step	Action
1	Open the service compartment lid to access the probing and transfer pumps.

Note:

The Probing pump is located to the left and the Transfer pump to the right.

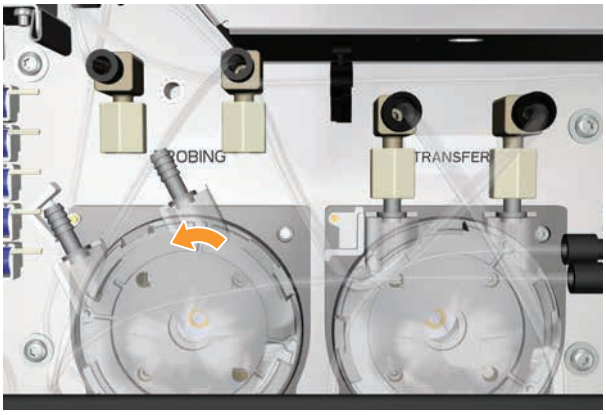
2	Disconnect the pump tubing on the left.

Step	Action
3	Disconnect the pump tubing on the right.



4	Unlock the catch that keeps the pump head in position.

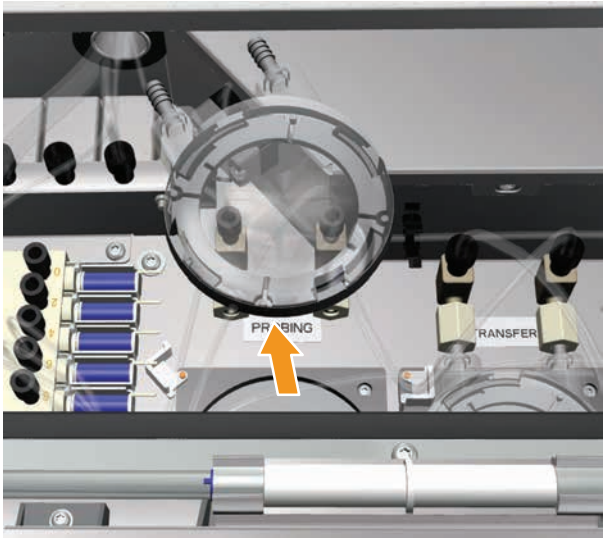
Step	Action
5	Turn the pump head counterclockwise.



6	Lift up the pump head out from the service compartment.

Note:

When removing the head be careful not to damage or bend any of the tubing in the Western unit. If any tubing is damaged or bent it needs to be replaced with new tubing. Contact Service for help.



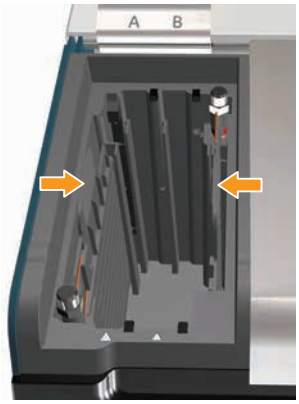
7	Unpack a new pump head.

Step	Action
8	Place the new pump head into the correct position and turn clockwise. ***Note:*** *Make sure the corresponding wheel of the pump head is in the correct position to fit the drive shaft of the pump motor.*
9	Lock the catch to keep the pump head in the correct position.
10	Connect the pump tubing on the right to the right connector of the pump head.
11	Connect the pump tubing on the left to the left connector of the pump head.
12	Close the service compartment lid.

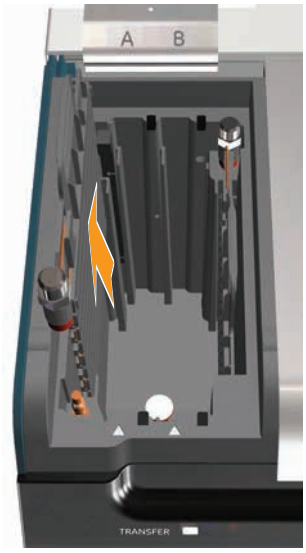
Replace transfer electrodes

If the transfer procedure fails, the reason could be defective transfer electrodes. It is recommended to replace both the electrodes. The instruction below describes how to replace the transfer electrodes.

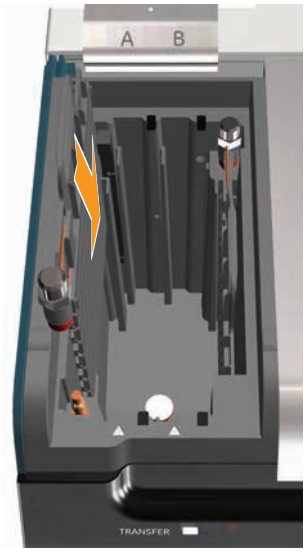
Step	Action
1	Open the transfer tank lid. The electrodes are located on each side.



Step	Action
2	Pull up the electrode.



3	Insert the new electrode and press down.



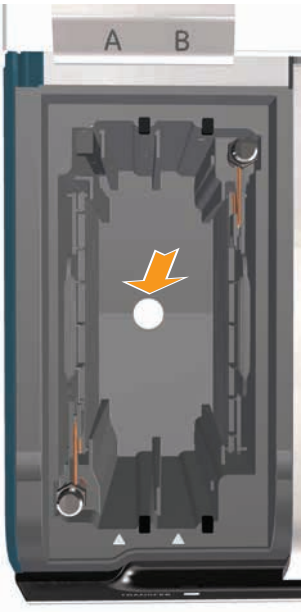
4	Repeat the procedure in the same way with the right side electrode.
5	Close the transfer tank lid.

Replace transfer tank filter

Note: *If the transfer filter is dirty, it is not possible to remove, clean and put it back again. The filter must be replaced.*

The instruction below describes how to replace the transfer tank filter.

Step	Action
1	Open the lid of the transfer tank. The filter is located in the bottom of the transfer tank.



2	Pierce through the middle of the filter using tweezers. ***Note:*** *Do not remove the filter by gripping its edges, as this might damage the filter seal.*
3	Use the hole to grip and remove the filter.
4	Place the new filter and push it gently, onto the filter seal.

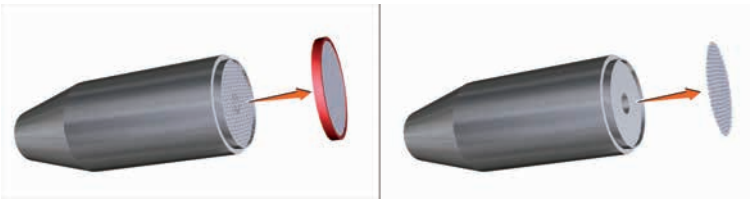
Replace the inlet filters

Replace the inlet filter when required, for example when the filters become clogged.

Required material: Inlet filter set.

Follow the instructions below to replace an inlet filter and a support net.

Step	Action
1	Pull off the inlet filter and the support net from the inlet filter holder.



2	Fit the new support net and inlet filter on the inlet filter holder, and press the filter into position.

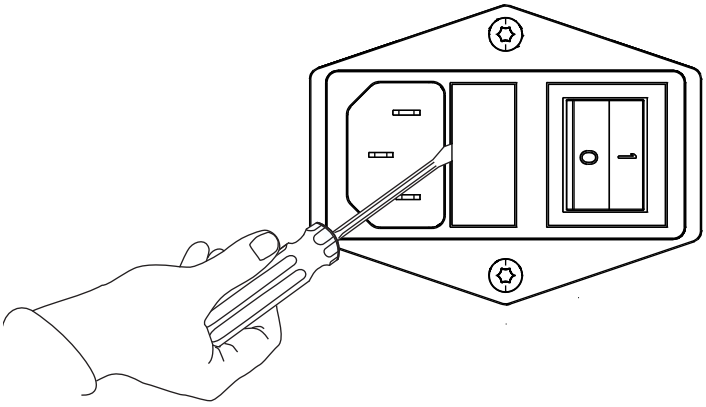
Replace the mains fuse

WARNING

Always disconnect the mains supply before replacing the mains fuse. For continued protection against risk of fire, replace only with a fuse of the same type and rating as stated on the instrument unit labels.

Step	Action
1	Turn off the power to the Amersham WB analyzer by setting the mains power buttons on the rear sides of the Elpho & scan and Western units to the off (**0**) position.
2	Disconnect the mains cords from the mains power inlets on the Elpho & scan and Western units.
3	Locate the the fuse drawer in the connector panel on the appropriate unit.

Step	Action
4	Insert a small screwdriver into the notch next to the fuse drawer.



5	Twist the screwdriver to open the fuse drawer.
6	Replace the fuses. For fuse data, see the instrument unit labels on the rear panels.
7	Insert the fuse drawer into its receptacle on the connector panel.

6.2.3 Moving the instrument units

Precautions

CAUTION

Personal Protective Equipment (PPE). Whenever packing, unpacking, transporting or moving the system, wear:

- Protective footwear, preferably with steel lining
- Working gloves, protecting against sharp edges
- Protective glasses

CAUTION

Heavy object. Two people are required to lift the instrument units safely.

CAUTION

Never move the Western unit with bottles standing on the unit.

NOTICE

Disconnect cables. To prevent equipment damage, always disconnect cables before an instrument unit is moved.

Lifting instruction

Step	Action
1	Disconnect all cords and cables from the instrument units and remove any bottles on the units.
2	Two people are required. To lift a unit, grip with both hands on each side of the unit and lift.

7 Troubleshooting

Introduction

This chapter provides general advice for troubleshooting for the Amersham WB system. For advice regarding electrophoresis and Western blotting in general, please consult the handbook *Western Blotting – Principles and Methods* 28-9998-97.

General

Problem	Possible cause and action
Red indicator is lit.	• **An error has caused the red indicator to be lit**. Open the software and read the instructions on the screen.
Loader cannot be closed.	• **The sealing lid is not closed properly**. Make sure that the sealing lid is closed.
Spots or clouds in the image	• **Contaminations** Always use clean powder-free gloves and only touch the frame when handling the gel card and PVDF card. Load the buffer strip by pushing it out from the package directly into the buffer strip holder. Make sure to use clean incubation trays that have not previously contained stains, such as Coomassie or fluorescent stains. Make sure that all equipment and components are clean and free of residues from a previous run. Make sure that recommended cleaning procedures have been performed, and in a correct way.
No contact with instrument	• **No connection** Check the USB cable and that the power is on for both the Elpho & scan unit and the Western unit. Unplug the USB cable and wait for 10 s until you reconnect the cable. This will restart the communication. If this does not work close the Amersham WB software and restart to get communication.

Pre-labeling

Problem	Possible cause and action
White precipitation in Labeling buffer. **White/orange precipitation in Loading buffer.**	• **SDS precipitates at low temperature.** Equilibrate the vials to room temperature and vortex.
The protein precipitates in Labeling buffer during sample preparation.	• **SDS is included in the Labeling buffer. Some proteins may precipitate when they come in contact with SDS.** Heat the sample before pre-labeling as described in the pre-labeling protocols.

Electrophoresis

Problem	Possible cause and action
Air between gel and cassette before run (visual appearance)	• **Expected behavior** The over pressure applied in the instrument will ensure all air is removed during the electrophoresis step. Performance is not affected.
Electrophoresis is not started due to no current through the gel.	• **Missing one or both buffer strips.** Remove the gel card and insert the missing buffer strip(s). Place a new gel card and reload samples. A gel card with the well lid removed cannot be reused.
The electrophoresis does not stop as expected. (Easy Western or Western with Endogenous protein normalization)	• **The front detection has not worked due to a weak front, when using no pre-labeled samples and no or too diluted MW markers.** Stop electrophoresis on time when using MW markers diluted higher than 20 fold.
Electrophoresis is not possible to re-start after power failure during a run.	• **The over-pressure has not been turned on.** Open the loader. Manually press down the sealing lid and then open and close one of the sealing lid latches. Close the loader and re-start the electrophoresis from the software.

Transfer

Problem	Possible cause and action
Transfer is not started	• **The transfer tank lid is not completely closed.** Make sure that the transfer tank lid is completely closed.
An error message during transfer: Too high resistance in transfer tank.	• **Mix up of tubing from transfer buffer and water**. Check buffer inlet. Empty the transfer tank and start a transfer with correct transfer buffer inlet. • **Transfer electrode malfunction** Remove the electrodes and check for damage. If damaged, see *Maintenance* chapter how to replace the electrodes.
Transfer tank is not emptied or filled in a correct way.	• **Clogged inlet filters of the transfer tubing** Clean or replace inlet filters, see *Maintenance* chapter regarding replacement. • **Clogged transfer filter in the bottom of the tank.** Carefully remove visual particles with tweezers after every run. A clogged filter must be replaced. To replace the filter, see *Maintenance* chapter.
Prompt for missing liquid even though the bottle is not empty.	• **Transfer pump malfunction** Replace the pump head. See *Maintenance* chapter. • **Leaking tubing connectors** Check all tubing connections in the flow path inside the Western unit.

Probing and drying

Problem	Possible cause and action
Antibody tubes are not emptied completely during probing.	• **Probing pump malfunction** Replace the pump head. See *Maintenance* chapter. • **Leaking tubing connectors** Check all tubing connections in the flow path inside the Western unit.

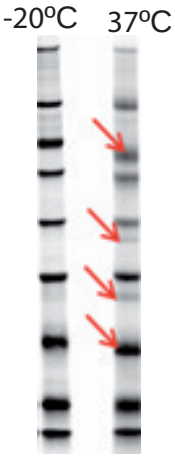
Problem	Possible cause and action
Antibody compartment is wet after cleaning of the probing flow path.	• **No empty antibody tubes connected** Always connect four empty tubes before cleaning is started.
Membrane is not dry after the drying procedure.	• **High humidity in lab** Repeat drying procedure. • **Air filter is dirty.** Replace the air filter, see *Maintenance* chapter • **Ventilation is blocked.** Check ventilation. Make sure that inlet and outlet ventilations are not blocked.

Scanning

Problem	Possible cause and action
Weak signals in gel or membrane image.	• **The scanning sensitivity has been automatically set too low because there is some spot/part with stronger signals in the image, or in the other position (A/B). The automatic scanning sensitivity always set the same scanning sensitivity for both positions**. Re-scan the membrane, or gel, using a higher scanning sensitivity (manually).

Gel and membrane images

Problem	Possible cause and action
Fluorescent spots in the gel image	• **Dirty protective glass in the Elpho & scan unit**. Clean the glass, see *Clean the Elpho & scan unit, on page 183*
Lighter areas in the gel image	• **Card plate not dry in the Elpho & scan unit** Remove the gel, wipe the plate dry with a lint-free cloth and then re-scan the gel.

Problem	Possible cause and action
Unclear MW marker bands or double bands	• **MW markers degradation. The markers are not stable over long time in room temperature. Double bands occur and others become wider. See illustration below.** Always use fresh MW marker solution and store at -20°C. 
M_r 10 000 or even the 14 000 band is missing in the MW marker lane.	• **The gel has run for too long time.** It is still possible to do a MW calibration with the remaining bands. Remove the missing band(s) in the ***Amersham WB MW markers*** scroll list, see *Section 5.5.4 Edit molecular weight calibration, on page 309*.

Problem	Possible cause and action
Diffuse bands, or poor band resolution	• **Too large sample volumes or too high protein concentration when loading on gel.** Follow the recommended loading 20 µl and max 20 µg. • **Loading buffer has not been used.** Use Loading buffer and prepare the sample according to recommendations. See *Section 4.4.2 Prepare unlabeled samples, on page 161*. • **Too high concentration of DTT** Decrease the DTT concentration to 20 mM (final). • **Loading buffer too old.** Prepare fresh solutions. ***Note:*** *See also the Troubleshooting chapter in the handbook Western Blotting – Principles and Methods 28-9998-97 for examples and solutions.*
Vertical streaks in lanes	• **Streaks in lower part of gel due to sample wells not cleaned** Select the option ***Pause for sample well cleanup*** before starting electrophoresis and use a paper comb to remove excess Cy5 dye reagent. • **Too high concentration of DTT** Decrease the DTT concentration to 20 mM (final)
Precipitations in the sample well or stacking	• **Protein samples with precipitation** During protein sample preparation, the samples should be mixed by vortexing and centrifuged before and after the heating step, prior to loading for best resolution.

Problem	Possible cause and action
Smeared bands	• **Samples are not fully reduced.** Add DTT to the Loading buffer according to recommendations (40mM). • **High salt concentrations.** Dialyze the sample, precipitate the protein with TCA or use desalting columns. • **Concentration of protein is too high when loading on gel.** Follow the recommendations in manual. Load no more than 20 µg per well.
No distinct lanes	• **Leakage between the gel card wells due to the sample well cover have been removed before the gel card was placed in the loader, or the sealing lid have been opened after the loading of samples.** It is important to close the sealing lid before removing the sample well cover.
Lanes are wider in the low molecular weight region.	• **All wells are not filled, causing smaller proteins in the sample wells to diffuse during run.** Fill unused wells with Loading buffer, diluted with an equal volume of ultra pure water.

Problem	Possible cause and action
No bands on the membrane	• **Transfer did not work.** Make sure that the foil is removed from the gel before transfer and that the gel is placed in the transfer holder before the PVDF card. Sandwich order from bottom: Sponge, Transfer paper, Gel, PVDF card, Transfer paper and Sponge. • **No antibody binding** Make sure to place the antibody tubes in correct positions in the antibody compartment. Do not use primary antibody species other than mouse or rabbit, and make sure the secondary antibodies are directed against the species in which the primary antibodies were raised. • **Insufficient membrane equilibration procedure** It is important to follow the recommendations in the manual. Be aware that the membrane shall never partly dry. Keep the membrane completely wet all the time to avoid drying before transfer and probing. • **Target protein not present at detectable levels.** Use positive control to test the blotting procedure. ***Note:*** *See also the Troubleshooting chapter in the handbook Western Blotting – Principles and Methods 28-9998-97 for examples and solutions.* *See also troubleshooting for weak bands below.*

Problem	Possible cause and action
Week bands on the membrane (general)	• **Wrong blocking agent: the blocking agent may have strong affinity for the protein of interest.** Try different blocking solutions and concentrations. • **Insufficient primary antibody incubation** Optimize primary antibody incubation time. • **Too low antibody concentration** Optimize antibody concentration. • **Weak or inactive antibody** Use a specific primary antibody with high affinity for the target protein, validated for Western blotting. • **Too low amount of target protein in the sample.** Concentrate the protein sample, but follow the recommended maximum protein load on the gel, 20 µg per well. ***Note:*** *See also the Troubleshooting chapter in the handbook Western Blotting – Principles and Methods 28-9998-97 for examples and solutions.*
Weak bands (poor transfer) for small proteins	• **Insufficient protein retention.** Optimize transfer time. Use a gradient gel to help retain small proteins during transfer. • **Too low ethanol/methanol concentration in transfer buffer to remove SDS. SDS can interfere with the binding of small molecular weight proteins to membranes**. Use a higher percentage of ethanol in transfer buffer (max. 40%). Do not use SDS in transfer buffer.
Weak band (poor transfer) for large proteins	• **Insufficient transfer time.** Increase transfer time to increase binding. • **Ethanol/Methanol concentration is too high.** Reduce ethanol concentration.

Problem	Possible cause and action
High background on the membrane	• **Insufficient quality of chemicals used in transfer and probing** Always use high quality chemicals. • **Ethanol with high auto fluorescence used in transfer** Some ethanol grades will give rise to a high background especially in Cy3. Make sure that the ethanol used during transfer is not auto fluorescent. • **Wrong detergent or incorrect concentrations are used in buffers.** Check that the correct detergent and concentrations are used • **Insufficient blocking** Make sure that appropriate blocking conditions are used. Use freshly prepared blocking agent that is fully dissolved. • **Insufficient washes** Increase the number of washing steps, volume or duration of the washing steps. ***Note:*** *See also the Troubleshooting chapter in the handbook Western Blotting – Principles and Methods 28-9998-97 for examples and solutions.*
Uneven background on the membrane	• **Membrane not completely dry** Dry the membrane again and re-scan. • **A felt pen or a pen has been used when marking consumables.** Always use a pencil when marking buffer strip packages, reagent packages, PVDF cards, gel cards, etc. ***Note:*** *See also the Troubleshooting chapter in the handbook Western Blotting – Principles and Methods 28-9998-97 for examples and solutions.* *See also troubleshooting for spots or clouds in the image.*

Problem	Possible cause and action
Uneven band intensity across membrane	• **Probing liquids do not cover the complete membrane during probing.** Western unit does not stand horizontally. Adjust the feet of the Western unit. • **Insufficient volume of antibody solutions** Increase the volumes. The recommended minimum volume of antibody solutions is 5 ml. • **Partly dry membrane before transfer or probing** It is important to follow the recommendations in the manual. Be aware that the membrane shall never partly dry. Keep the membrane completely wet all the time to avoid drying before transfer and probing. • **The antibody has not been pumped correctly.** See the problem "Antibody tubes are not emptied completely during probing" in the *Probing* section above.
Clouded bands, bands spread out into swirls or white spots on the membrane	• **Insufficient contact between membrane and gel during transfer** Make sure the transfer sandwich is assembled correctly according to the instructions.
Black fluorescent spots on the membrane / Speckled background	• **Dirty protective glass** Clean the glass with a lint-free cloth. • Dirty membrane adapter Clean the membrane adapter, using a lint-free cloth. • Aggregates in the blocking agent Make sure the blocking agent is completely dissolved in the buffer. ***Note:*** *See also troubleshooting for spots or clouds in the image.*
The membrane overlay image displays bad alignment between the two images (Cy3 and Cy5 scans).	• **The membrane has been scanned for Cy3 and Cy5 at different occasions. Slightly different positions of membranes during scanning.** Always scan both channels at the same time.

Problem	Possible cause and action
Multiple bands of small proteins using the SDS-PAGE pre-labeling protocol	• **Too many dye molecules per labeled protein molecule can give rise to multiple bands for small proteins. The extra bands appear with higher Mw and weaker intensity compared to the main band.** Use less dye in the SDS-PAGE pre-labeling reaction. This can be achieved by decreasing the volume of dye reagent or diluting the dye in DMSO, e.g. by a factor in the range 2-5. An other option is to scale up the reaction and keep the amount of dye constant.

Evaluation

Problem	Possible cause and action
Some options in Evaluation are disabled or unavailable, for example Quantity calibration.	• **Incorrect Experiment type in the start screen or incorrect Sample types in the experimental setup have been chosen.** Sample types can be changed after the run. If wrong Experiment type, create a new experiment of correct type, re-scan the gel and/or membranes and perform evaluation.
Multiple images using different sensitivities have been scanned but only one of them is visible in Evaluation.	• **The different images are visible in the image stack in the scanning screens. The image that is selected is the one that is used in Evaluation.** Select the preferred image in the scanning screens in order to use it in Evaluation.
The Normalized ratios for Quantitative Western blotting varies significantly from run to run.	• **Automatic scanning sensitivity may result in different sensitivity settings between runs. Different sensitivity settings will directly affect the ratio between target and control signals**. Only compare normalized ratios within the same run using the same sensitivity settings. Use manual sensitivity setting when scanning.

8 Reference information

About this chapter

This chapter lists the technical specifications of the Amersham WB system. The chapter also includes a chemical resistance guide and a decontamination report that must be used to record decontamination details before a service.

In this chapter

This chapter contains the following sections:

8.1 System specifications

Introduction

This section lists the system specification data of the Amersham WB analyzer.

Environmental ranges

Parameter	Data
Storage and transport temperature range	-25°C to +60°C
Chemical environment	See *Section 8.2 Chemical resistance guide, on page 358*.

Operating range

Parameter	Limits
Operating temperature range	15°C to 32°C For full performance: 16°C to 28°C
Relative humidity	20% to 80%, non-condensing For full performance: 20% to 70%, non-condensing
Altitude	Maximum 2000 m
Pollution degree	2
Transient level	Overvoltage category II
Environment	Indoor use only
EMC	EN 61326-1, IEC61326-1 and FCC Part15B. (Emission according to CISPR 11, Group 1, class B)

System specifications Amersham WB analyzer

Parameter	Data
System configuration	Benchtop system consisting of external computer and two units: Elpho & scan unit and Western unit The computer is not included in the delivery.
Controlling computer operating system	PC with Windows 7
Control software	Amersham WB software
Connection between Elpho & scan and Western units	Ethernet cable
Connection between PC and Elpho & scan unit	USB cable (Type A to Type B)
Pollution degree	2
Sound level	Below 80 dB(A)

System specifications Elpho & scan unit

Parameter	Data
Dimensions (W x D x H)	47 x 51 x 27 cm
Weight (excluding computer)	25 kg
Power supply	Voltage: 100-240 V~ Frequency: 50-60 Hz
Power consumption	Max Power: 300 VA
Fuse	2x T4AH 250 V

Scanner specifications

Parameter	Data
Image sensor	Silicon Photodiode
Warm up time	At least 1 minute ***Note:*** *The warm up is included in the automatic sensitivity mode.*
Lens	F1.0/13 mm
Light source	Cy5: Laser diode module, 635 nm, 10 mW
	Cy3: Laser diode module, 532 nm, 10 mW.
Operation	Fully automated (auto exposure, no focus or other adjustment or calibrations needed)
Maximum sample size	2 samples of approximately 80 x 65 mm^2
Gray scale	65 536 levels (16 bit)
Dynamic range	4.6 orders of magnitude
Image output	Gray scale 16 bit (tif)

Separation specifications

Parameter	Description
Voltage	250-600 V [1]
Current	20-50 mA [1]

1 Maximum power is 20 W/gel card. The maximum values for the parameters can not be reached simultaneously.

System specifications Western unit

Parameter	Data
Dimensions (W x D x H)	43 x 53 x 39 cm
Weight	20 kg
Power supply	Voltage: 100-240 V~ Frequency: 50-60 Hz
Power consumption	Max Power: 400 VA
Fuse	2x T4AH 250 V
Transfer tubing and connectors	Tubing material: FEP, ID 1/8" Ferrule blue Tubing connector: Nut 5/16"-24UNF 2-A
Probing tubing and connectors	Tubing material: FEP, ID 0.063" Ferrule yellow Tubing connector: Nut 1/4"-28UNF 2-B

Transfer specifications

Parameter	Description
Voltage	10-100 V [1]

1 Maximum power is 40 W. Maximum current is 400 mA.

Probing specifications

Parameter	Description
Volumes, antibody probing	5-12 ml

Drying specifications

Parameter	Description
Temperature	Maximum 45°C
Drying time	10 minutes

8.2 Chemical resistance guide

Introduction

This section specifies the chemical resistance of Amersham WB analyzer to some of the most commonly used chemicals in electrophoresis and Western blotting.

Assumptions made

The ratings are based on the following assumptions:

- Synergy effects of chemical mixtures have not been taken into account.
- Room temperature and limited overpressure is assumed.

Note: *Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.*

List of chemicals

For a list of chemicals used in the gel cards and buffer strips, see the Safety Data Sheet (SDS/MSDS).

Usage	Chemical	Concentration	CAS no and EC no
General	Aqueous buffers pH 4-10 (e.g., Tris, Glycine, Phosphate)	0-0.2 M	N/A
Transfer	Ethanol	40%	75-08-1/200-837-3
	Methanol	40%	67-56-1/200-659-6
Transfer & Probing	Sodium Chloride	0.2 M	7647-14-5/231-598-3
	Potassium Chloride	50 mM	7447-40-7/231-211-8
	Tween	1%	9005-64-5/500-018-3
Cleaning of flow paths	Sodium Hydroxide	0.5 M	1310-73-2/215-185-5
	Sodium Hypochlorite	5%	7681-52-9/231-668-3
Cleaning of card plate	Ethanol	50%	75-08-1/200-837-3

Usage	Chemical	Concentration	CAS no and EC no
Cleaning of surfaces	Ethanol	96%	75-08-1/200-837-3
	Mild detergents	N/A	N/A

9 Ordering information

Introduction

This chapter lists the ordering information for consumables, accessories, spare parts for user maintenance etc. For the most recent information, refer to www.gelifesciences.com.

Consumables

The table below lists the different consumables that can be ordered. Depending on the experiments to be performed, different combinations of consumables are required. A selection guide for putting a kit of consumables together is available at www.gelifesciences.com.

Consumable	Product code
Amersham WB Cy5	29-0307-31
Amersham WB labeling buffer	29-0307-32
Amersham WB loading buffer	29-0307-33
Amersham WB molecular weight markers	29-0307-35
Amersham WB gel card 14, 8-18%	29-0225-65
Amersham WB gel card 14, 13.5%	29-0225-64
Amersham WB buffer strip	29-0225-70
Amersham WB PVDF card	29-0225-66
Amersham WB transfer paper	29-0639-02
Amersham WB goat anti-mouse Cy3	29-0382-75
Amersham WB goat anti-rabbit Cy3	29-0382-76
Amersham WB goat anti-mouse Cy5	29-0382-77
Amersham WB goat anti-rabbit Cy5	29-0382-78
Amersham WB paper comb	29-0562-86

Accessories

Accessory	Product code
Amersham WB buffer strip holder	29-0479-61
Amersham WB transfer holder	29-0384-64
Amersham WB sponge	29-0341-13
Amersham WB drying holder	29-0993-46
Amersham WB membrane adapter	29-0384-66
Barcode scanner 2-D with USB	28-9564-52
Tubing holder	29-0562-62
Inlet filter holding kit: • 1 x Filter holder • 1 x Filter • 1 x Support screen	11-0004-07
Inlet filter set: • 5 x Filter • 5 x Support screen	11-0004-14

Spare parts for user maintenance

Spare part	Product code
Transfer electrode	29-0384-65
Air filter (dryer)	29-0341-15
Transfer tank filter	29-0454-13
Pump head (same head for transfer and probing pump)	29-0341-14
Fuse: 2x T4AH 250 V	22-0659-27

Product recommended for buffer exchange

Buffer exchange product	Product code
Amersham WB MiniTrap kit	29-0222-21

Handbook

Handbook	Product code
Western Blotting – Principles and Methods	28-9998-97

Instrument

Product	Product code
Amersham WB analyzer	29-0320-30

Appendix A The Amersham WB watch app

Introduction

The instrument status and the progress of runs can be remotely monitored through the Amersham WB watch app. The app can alert users when the instrument requires manual intervention or when runs are completed. Optionally snapshots of the images from the last run can be previewed. No detailed run information like e.g., file names, sample names and notes will be possible to view remotely.

It is also possible to select whether to upload service-related data to the cloud for service and maintenance purposes.

To establish connection between an instrument and a registered user account in the mobile device, follow the steps below:

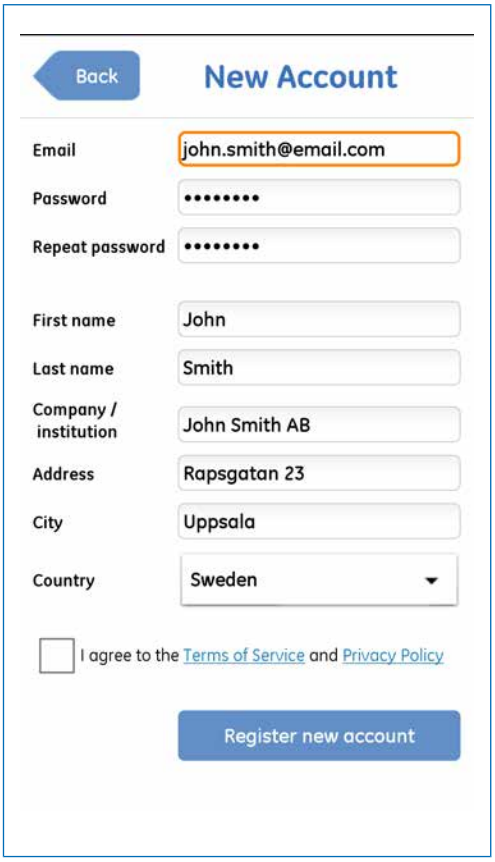
- install the app and register a user account
- activate an instrument for remote access
- add the instrument to the app by entering an access code in the app (the access code is obtained from the software connected to the instrument)

Note: *In order for the Amersham WB watch app status updates to work, the computer on which the Amersham WB software is installed:*

- *must be connected to the instrument and have Internet access*
- *may not be in power save mode (sleep or hibernate)*

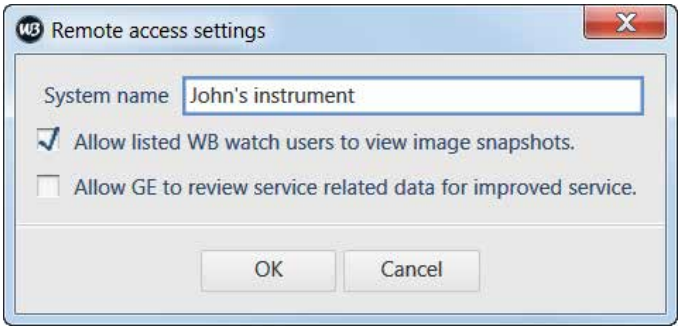
Install Amersham WB watch and register an account

Step	Action
1	Download Amersham WB watch from Apple™ AppStore or Google Play™ and install it.
2	Select ***Register new account*** in the app, fill out the registration form and click the ***Register new account*** button.



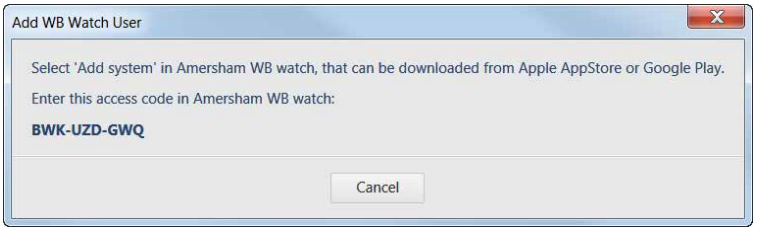
Result: The user account is registered.

Activate instrument remote access

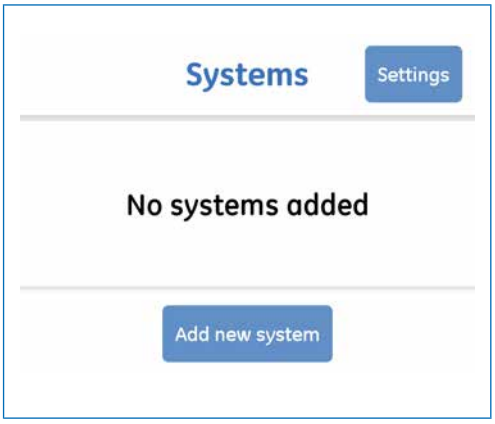
Step	Action
1	Select ***Remote:Activate remote access*** in the menu bar in Amersham WB software to enable remote access to Amersham WB analyzer. ***Note:*** *The **Remote** menu is only available when connected to an instrument.* *Result:* If it is the first time that the connection is established to the instrument, the ***Remote access settings*** dialog opens.
2	If appropriate, edit the alias for the instrument in the ***System name*** field (default is the serial number of the instrument). This name will appear in the app for users with remote access to the instrument. 
3	Check the ***Allow listed WB watch users to view image snapshots*** box to allow snapshots of the last scanned images to be uploaded to the cloud. All users connected to the system will be able to see the images.
4	Check the ***Allow GE to review service related data for improved service*** box to allow service-related data to be uploaded to the cloud for service and maintenance purposes.

Add a WB watch user

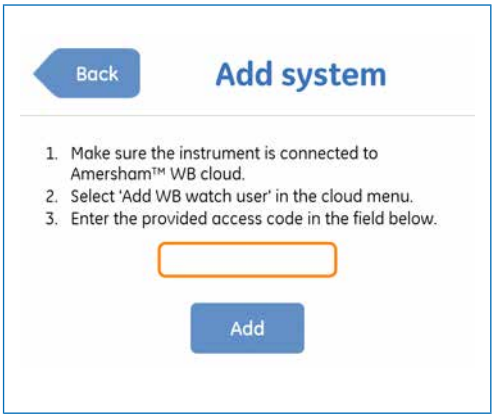
To add a WB watch user who will be able to monitor an instrument activated for remote access:

Step	Action
1	Select ***Remote:Add WB watch user*** in the menu bar of the software connected to the activated instrument. ***Note:*** *This menu option is only available when connected to an instrument with remote access activated.* *Result:* The ***Add WB Watch User*** dialog opens displaying an access code for the user to enter in the Amersham WB watch app to establish the remote connection. ***Note:*** *The access code can only be used once.* 

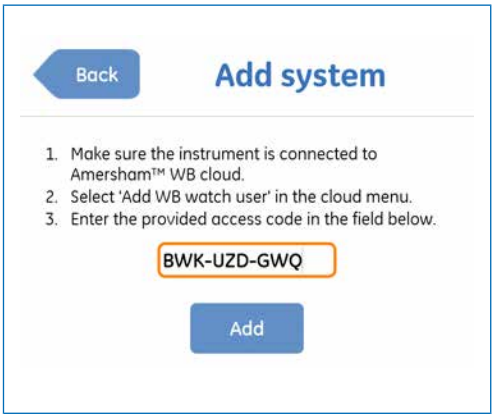
Step	Action
2	Select ***Add new system*** in the Amersham WB watch app.



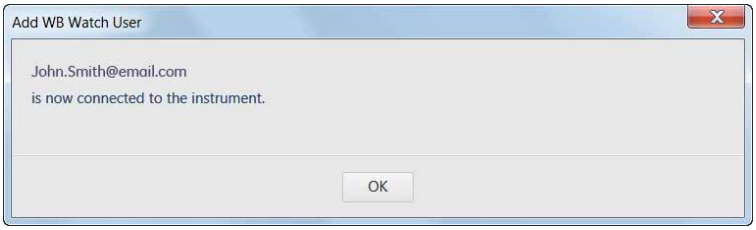
Result: The ***Add System*** page is displayed.



Step	Action
3	Enter the access code from ***Add WB Watch User*** dialog (in the Amersham WB software) in the ***Access Code*** text field and select ***Add***.

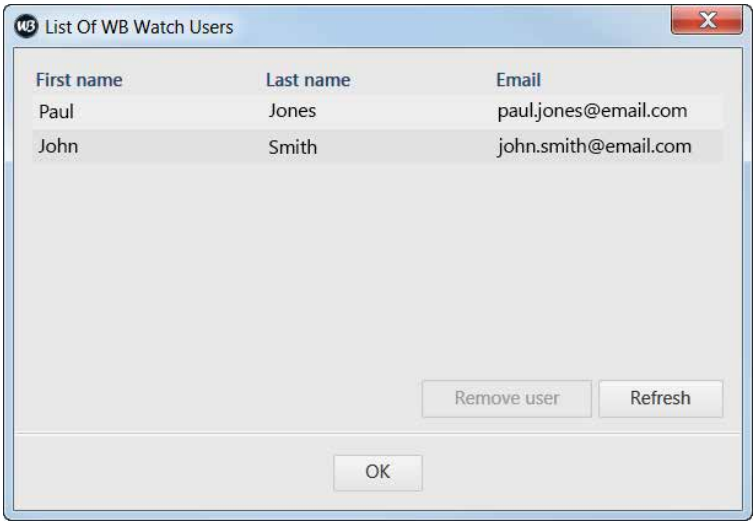


Result: The added instrument appears in the app and the ***Add WB Watch User*** dialog in the software displays the e-mail address of the added user.



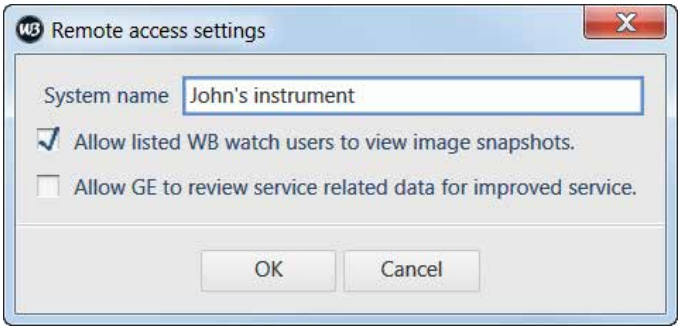
Remove a user

To remove a user from being able to remotely monitor an instrument:

Step	Action
1	Select ***Remote:List of WB watch users*** in the menu bar. *Result:* The ***List Of WB Watch Users*** dialog opens. 
2	Select the name of the user to be removed.
3	Click the ***Remove user*** button.
4	Click ***OK*** to close the dialog.

Edit remote access settings

To edit the system name or change the type of data to be uploaded to the cloud:

Step	Action
1	Select ***Remote:Remote access settings*** in the menu bar. *Result:* The ***Remote access settings*** dialog opens. 
2	The following can be edited: • the alias for the system in the ***System name*** field (visible in the app). • check/clear the ***Allow listed WB watch users to view image snapshots*** box. When checked, snapshots of the last scanned images are uploaded to the cloud and are available for connected users. ***Note:*** *Status data for the run will be shown to all WB watch users regardless of these options. To stop a user from viewing status, remove the user. See Remove a user, on page 369 above.* • check/clear the ***Allow GE to review service related data for improved service*** box. When checked, service related data is uploaded to the cloud for service and maintenance purposes.

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