Amersham™ ECL™ start Western Blotting Detection Reagent

Product booklet

Code: RPN3243 RPN3244 RPN3245



Page Finder

1.	Lega		4
2.	Desc 2.1 2.2 2.3	5	5 5 6 6
3.	Impo 3.1 3.2 3.3	Safety notices	7 7 7 7 7
4.	Hand 4.1 4.2 4.3	Safety precautions	8 8 8 8 8
5.	Requ 5.1 5.2 5.3 5.4 5.5	Solutions Membrane	9 9 9 10 10
6.	West 6.1 6.2 6.3 6.4 6.5 6.6	ern blotting optimization Molecular weight markers Membranes Transfer Blocking Imagers and film Western blotting handbook	11 11 12 12 12 13 14
7.	Proto 7.1	col Electrophoresis and transfer	15 16

	7.2	Blocking	16
	7.3	Antibody probing	16
	7.4	Dilution ranges	17
	7.5	Primary antibody incubation	17
	7.6	Secondary antibody incubation	17
	7.7	Streptavidin bridge incubation	18
	7.8	Detection	18
	7.9	Image analysis	19
	7.10	CCD camera	19
	7.11	X-ray film	20
8.	Additio	onal information	21
	8.1	Stripping and reprobing membranes	21
	8.2	Determination of optimum antibody concentration	22
	8.3	Dilution ranges	22
	8.4	Primary antibody optimization	22
	8.5	Secondary antibody optimization	24
9.	Troub	leshooting guide	25
10.	Relate	d products	28
	10.1	Sample preparation	28
	10.2	Molecular weight markers	28
	10.3	Gel electrophoresis equipment	29
	10.4	Transfer equipment	30
	10.5	Blotting papers and membranes	31
	10.6	Blocking agents	32
	10.7	Amersham ECL HRP-linked secondary antibodies	32
	10.8	Detection reagents	33
	10.9	X-ray films	34
	10.10	Imaging systems	34
	10.11	Software and accessories	34

1. Legal

GE, GE monogram and imagination at work are trademarks of General Electric Company.

Amersham, ECL, ECL DualVue, ECL Select, Hybond, Hyperfilm, ImageQuant and Rainbow are trademarks of General Electric Company or one of its subsidiaries.

Amersham ECL start is manufactured and sold under license from Cyanagen Srl and is subject of US Canadian and EU patent application number EP1950207A1, US2008176251A1, ITB020070037A1, EP1962095A1, US2008241868A1, ITB020070112A1, together with other equivalent granted patents

and patent applications in other countries.

Tween is a trademark of ICI Americas Inc.

All other third party trademarks are the property of their respective owner.

© 2014 General Electric Company – All rights reserved. First published October 2014

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

http://www.gelifesciences.com/ecl

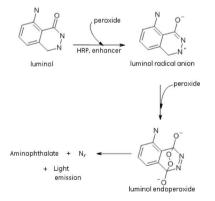
GE Healthcare UK Limited. Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA UK

2. Description

Amersham ECL start Western blotting detection reagent is an entrylevel chemiluminescent detection reagent suitable for detection of high to medium expressed proteins.

2.1 Introduction

Chemiluminescence is defined as light emission produced in a multistep reaction whereby peroxidase catalyzes the oxidation of luminol. In the presence of chemical enhancers and catalysts, the light intensity and the duration of light emission is greatly increased in a process known as enhanced chemiluminescence (ECL). ECL based on horseradish peroxidase (HRP)-conjugated secondary antibodies is a sensitive detection method where the light emission is proportional to protein quantity. The multi-step reaction is illustrated below.



2.2 Design and features

Amersham ECL start Western blotting detection reagent is designed to provide high light output. The high signal intensity can fully utilize the advantages of CCD camera based imaging equipment and makes Amersham ECL start optimal for detection with ImageQuant LAS 500 and Amersham Imager 600 from GE Healthcare. The output light can also be detected using X-ray film (Amersham Hyperfilm[™] product range).

2.3 Compatibility

Amersham ECL start Western blotting detection reagent is compatible with PVDF and nitrocellulose membranes (such as Amersham Hybond[™] and Amersham Protran product range), Amersham ECL Prime Blocking Agent, Amersham ECL Blocking Agent and other commonly used blocking agents such as BSA and non-fat dry milk.

3. Important user information

3.1 Intended use

Amersham ECL start Western blotting detection reagent is intended for chemiluminescent detection in Western blotting.

Amersham ECL start Western blotting detection reagent is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

3.2 Safety notices

This user documentation contains CAUTIONS concerning the safe use of Amersham ECL start Western blotting detection reagent. See definitions below.

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.

3.3 Quality control

Amersham ECL start Western blotting detection reagent is manufactured in compliance with our ISO 9001 certified quality management system, and is in conformity with the acceptance criteria set up for the product.

4. Handling

4.1 Safety precautions

CAUTION

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation.

It is recommended to read the Safety Data Sheet (SDS) before using Amersham ECL start Western blotting detection reagent.

4.2 Storage

On receipt, all components should be stored in a refrigerator at 2°C to 8°C. Amersham ECL start Western blotting detection reagent is sensitive to prolonged exposure to light. Always store the individual reagents in the light-tight containers in which they are provided.

4.3 Expiry

The components are stable for at least 3 months when stored under the recommended conditions. See expiry date on package.

5. Required components

5.1 Kit components

The following components are included in the Amersham ECL start Western blotting detection reagent kit.

Product	Content
RPN3243	 Solution A: Luminol solution, 100 ml Solution B: Peroxide solution, 100 ml Sufficient for 2000 cm² membrane
RPN3244	 Solution A: Luminol solution, 200 ml Solution B: Peroxide solution, 200 ml Sufficient for 4000 cm² membrane
RPN3245	 Solution A: Luminol solution, 10 ml Solution B: Peroxide solution, 10 ml Sufficient for 200 cm² membrane

5.2 Solutions

Required solutions are listed below.

- Phosphate buffered saline (PBS), pH 7.5
- Tris buffered saline (TBS), pH 7.6
- Dilution and wash buffer: PBS Tween™ (PBS-T) and TBS Tween (TBS-T). A Tween 20 concentration of 0.1% is suitable for most blotting applications.

5.3 Membrane

Use a suitable protocol to separate proteins by electrophoresis and transfer them to a PVDF or nitrocellulose membrane.

5.4 Blocking reagents

Blocking reagents are typically diluted to 2% to 5% (v/v) in PBS-T or TBS-T buffer. The following blocking reagents are recommended:

- Amersham ECL Prime Blocking Agent
- Amersham ECL Blocking Agent
- Non-fat dry milk
- Bovine Serum Albumin (BSA)

5.5 Immunodetection reagents

- Primary antibody specific to the target protein(s)
- HRP conjugated secondary antibody specific to the primary antibody. See ECL HRP-linked secondary antibodies, on page 32.

Dilute the antibodies in PBS-T or TBS-T according to the recommendations in *Dilution ranges, on page 17.*

6. Western blotting optimization

Introduction

To achieve an optimal Western blotting result with high signal to noise ratio and best possible sensitivity and linearity, it is important to optimize the method and to select compatible products.

Consider the following:

- Sample quality and loading amount It is important that the sample is of good quality and that detectable levels of target protein are present.
- Membrane and blocking Select membranes and blocking agents compatible with sample and antibodies.
- Primary and secondary antibodies Always select specific antibodies of high quality and optimize the antibody dilution.
- Detection and imaging Select detection reagent according to your application need. A CCD imager may offer high sensitivity and broad dynamic range and provide better quantification than X-ray film.

This chapter describes products recommended for use with Amersham ECL start Western blotting detection reagent. For more information regarding these products, refer to <u>www.gelifesciences.com/ecl</u>

6.1 Molecular weight markers

Molecular weight markers are used to determine protein size. In addition, pre-stained markers allow confirmation of protein transfer and orientation (as the colored bands transfer to the membrane).

• Amersham ECL Rainbow™ Markers are pre-stained multicolored markers for monitoring progress of protein electrophoresis,

confirming transfer efficiency and determination of molecular weight of blotted proteins.

 Amersham ECL DualVue™ Markers are markers optimized for use with Amersham ECL start, Amersham ECL, Amersham ECL Prime and Amersham ECL Select™ and contains a combination of pre-stained and tagged proteins markers. These markers enable monitoring of electrophoresis, confirming transfer efficiency and determination of molecular weight of blotted proteins without staining on gel and membrane, as well as in chemiluminescence detection.

6.2 Membranes

- Amersham Hybond PVDF membranes have protein binding capacity and mechanical strength, which makes them ideal for Western blotting applications where stripping and re-probing are needed. The membranes are optimal for use with Amersham ECL start, Amersham ECL, Amersham ECL Prime and Amersham ECL Select detection reagents.
- Amersham Protran are nitrocellulose membranes compatible with all chemiluminescent Western blotting substrates. The main advantage is the normally low background.

6.3 Transfer

- Wet transfer is the most commonly used transfer method. It provides efficient transfer of small to large proteins.
- Semi-dry transfer is faster than wet transfer and consumes less buffer. Semi-dry transfer works well for most proteins but transfer may be less efficient for large proteins. It might have reduced sensitivity for very low abundance proteins.

6.4 Blocking

After protein transfer, the membrane needs to be incubated in a blocking solution to prevent non-specific binding of antibodies,

which can increase background and non-specific protein bands on the blot. The blocking agent should be optimized for best results. No single blocking agent is optimal for all proteins and antibodies. GE recommend the following blocking agents compatible with Amersham ECL start, Amersham ECL, Amersham ECL Prime and Amersham ECL Select:

- Amersham ECL Prime Blocking Agent
- Amersham ECL Blocking Agent
- BSA Blocking Agent
- Non-fat dry milk

6.5 Imagers and film

The ImageQuant LAS 500 and Amersham Imager 600 are flexible systems that cover a wide range of imaging applications. These CCD based imagers provide an affordable option for protein detection with genuine benefits, including quantitation with high sensitivity, publication grade data, and image and data archiving.





ImageQuant LAS 500

Amersham Imager 600

Amersham Hyperfilm product range are trusted products for sensitive, qualitative chemiluminescent detection in Western blots.

6.6 Western blotting handbook

More technical help, tips and best practices can be found in the handbook Western Blotting Principles and Methods from GE (code no. 28-9998-97).

7. Protocol

Protocol overview

Below is an overview of the Western blotting detection protocol.



7.1 Electrophoresis and transfer

Step	Action
1.	Perform electrophoresis and transfer proteins to a suitable membrane according to standard protocols. Blots are preferably used immediately but may be stored in PBS-T or TBS-T at 2°C to 8°C. Note: Amersham Hybond P membrane should be pre-wetted in 100% methanol/ethanol prior to equilibration in transfer buffer.

7.2 Blocking

Step	Action
1.	Incubate the membrane in a suitable blocking solution on an orbital shaker for 1 hour at room temperature or overnight at 2°C to 8°C.
2	Briefly rinse the membrane with two changes of wash buffer. Note: For preparation of wash buffer, see Solutions, on page 9.

7.3 Antibody probing

Amersham ECL start Western blotting detection reagent is an entry level detection reagent and optimal antibody dilution varies between antibodies depending on affinity and quality.

Optimization of the antibody dilution can be performed by dot blot analysis (see *Determination of optimum antibody concentration, on page 22*).

7.4 Dilution ranges

The following dilution ranges are recommended:

Antibody	Dilution range from 1 mg/ml stock solution
Primary	1:500-1:3000
Secondary	1:5000-1:50 000

7.5 Primary antibody incubation

Step	Action	
1.	Dilute the primary antibody in PBS-T or TBS-T.	
2.	Incubate the membrane in the primary antibody solution on an orbital shaker for 1 hour at room temperature or overnight at 2°C to 8°C.	
3.	Briefly rinse the membrane with two changes of wash buffer.	
4.	Wash the membrane 4 to 6 times in wash buffer for 5 minutes each at room temperature on an orbital shaker.	

7.6 Secondary antibody incubation

Step	Action
1.	Dilute the secondary antibody (HRP conjugated or
	biotinylated antibody) in PBS-T or TBS-T.
2.	Incubate the membrane in the secondary antibody solution for 1 hour at room temperature on an orbital shaker.
3.	Briefly rinse the membrane with two changes of wash buffer.
4.	Wash the membrane 4 to 6 times in wash buffer for 5 minutes each at room temperature on an orbital shaker.

If using an HRP conjugated secondary antibody, proceed directly to 7.8 Detection.

If using a biotinylated antibody, proceed with the streptavidin bridge incubation protocol in 7.7 *Streptavidin bridge incubation*.

7.7 Streptavidin bridge incubation

Step	Action	
1.	Dilute the Streptavidin HRP conjugate or Streptavidin biotinylated HRP complex in PBS-T or TBS-T.	
2.	Incubate the membrane in the diluted solution for 1 hour at room temperature on an orbital shaker.	
3.	Briefly rinse the membrane with two changes of wash buffer.	
4.	Wash the membrane by suspending it in enough wash buffer to cover the membrane and agitate for 5 minutes at room temperature. Replace wash buffer at least 4 to 6 times.	

7.8 Detection

Step	Action	
1.	Prior to use, allow aliquots of the detection solutions to equilibrate to room temperature for 20 minutes.	
2.	Mix detection solutions A (luminol) and B (peroxide) in a ratio of 1:1 to a form a working solution of detection reagent. The final volume of working solution required is 0.1 ml/cm ² membrane. Note: If the mixed reagent is not to be used immediately, protect it from exposure to the light by either wrapping in foil or storing in a dark place.	

- 3. Drain the excess wash buffer from the washed membrane and place it protein side up in a suitable box or on a sheet of plastic wrap or other suitable clean surface. Add working solution onto the membrane, making sure it completely covers the membrane.
- 4. Incubate for 90 seconds at room temperature.
- 5. Drain off excess detection reagent by holding the membrane edge gently against a tissue.

7.9 Image analysis

Two protocols for image analysis are described, one for CCD camera based imaging and another for detection with X-Ray film.

7.10 CCD camera

Step	Action	Notes
1.	Place the blot, protein side up on a sample tray.	The blot can be placed on a piece of plastic wrap, protein side up, to facilitate easy movement of the film on the sample tray.
2.	Place the sample tray in the CCD camera compartment and select suitable exposure time and/or function.	Use the automatic exposure function or select exposure time manually. Recommended starting exposure time is 60 seconds. Increase or decrease exposure time depending on the



obtained signal intensity.

7.11 X-ray film

Step	Action	Notes
1.	Place the blot with protein side down on to a fresh piece of plastic and wrap in a single layer to minimize drying. Take care to gently smooth out any air bubbles on the protein side of the blot.	
2.	Place the wrapped blot with protein side up in an X-ray film cassette.	Make sure there is no free detection reagent in the cassette; the film must not get wet.
3.	Place a sheet of X-ray film (Amersham Hyperfilm product range) on top of the membrane. Close the cassette and allow exposure. Suitable exposure start time is 30 seconds.	This stage should be carried out in a dark room using red safe lights. Do not move the film while it is being exposed.
4.	Develop the film without delay. If necessary, on the basis of signal intensity obtained, estimate exposure time for a second sheet of film.	

8. Additional information

8.1 Stripping and reprobing membranes

The complete removal of primary and secondary antibodies from the membrane is possible following the protocol outlined below. The membranes may be stripped and reprobed several times.

Step	Action	Notes
1.	Place the membrane in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7) and incubate at 50°C for 30 minutes with occasional agitation.	If more stringent conditions are required, the incubation can be performed at 70°C or for a longer time.
2.	Wash the membrane for 3 × 10 minutes in PBS-T or TBS-T at room temperature using generous volumes of wash buffer. The membrane may be used immediately or stored in PBS-T or TBS-T at 2 to 8°C.	Membranes may be incubated with Amersham ECL start Western blotting detection reagent and exposed to film to ensure removal of antibodies.
3.	Block the membrane in a suitable blocking solution for 1 hour at room temperature.	
4.	Repeat the immunodetection protocol in Chapter 7, Protocol from Antibody probing to Image analysis.	

8.2 Determination of optimum antibody concentration

Optimal antibody dilutions may vary between different applications and depend on the quality and affinity for the target protein. Optimization of antibody concentrations is recommended to ensure the best results. Outlined below are protocols for determining optimal antibody concentrations.

8.3 Dilution ranges

The following dilution ranges are recommended:

Antibody	Dilution range from 1 mg/ml stock solution	
Primary	1:500-1:3000	
Secondary	1:5000-1:50 000	

8.4 Primary antibody optimization

Dot blotting is a quick and effective method of determining the optimum dilution of a primary antibody of unknown concentration. Alternatively, a Western blot can be prepared and then cut into several strips. It should be noted that some antibodies may require alternative blocking and washing steps to the ones suggested below.

Step Action

 Spot different amounts of protein sample, preferably a dilution series, on to a PVDF or nitrocellulose membrane and allow to air dry. Alternatively, use a dot blot or slot blot manifold. Make one sample series for each dilution to be tested.

> **Note:** PVDF membranes must be pre-wetted in methanol/ ethanol.

Step	Action
2.	Incubate in blocking solution for 1 hour at room temperature with agitation.
3.	Rinse the membranes briefly with two changes of wash buffer.
4.	Cut the membrane to get each sample series on a separate membrane strip.
5.	Prepare different primary antibody solutions within the recommended antibody range. Incubate each membrane strip in antibody solution for 1 hour at room temperature with agitation.
6.	Briefly rinse the membrane with two changes of wash buffer. Wash the membrane by suspending it in wash buffer and agitate for 5 minutes at room temperature. Replace wash buffer at least 4 to 6 times.
7.	Dilute the secondary antibody (using only one concentration) and incubate the membranes for 1 hour at room temperature with agitation.
8.	Rinse blots with two changes of wash buffer, then wash 4 to 6 times in fresh changes of wash buffer.
9.	Detect using Amersham ECL start Western blotting detection reagent detailed in <i>Detection, on page 18</i> of the protocol. The antibody dilution which gives the best signal with the minimum background should be selected.

8.5 Secondary antibody optimization

Step Action

 Spot different amounts of protein sample, preferably a dilution series, on to a PVDF or nitrocellulose membrane and allow to air dry. Alternatively, use a dot blot or slot blot manifold. Make one sample series for each dilution to be tested. Note: PVDF membranes must be pre-wetted in methanol/ethanol.

- 2. Incubate in blocking solution for 1 hour at room temperature with agitation.
- Incubate in diluted primary antibody (optimized concentration) for 1 hour at room temperature with agitation.
- 4. Briefly rinse the membrane with two changes of wash buffer. Wash the membrane by suspending it in wash buffer and agitate for 5 minutes at room temperature. Replace wash buffer at least 4 to 6 times.
- Cut the membrane to get each sample series on a separate membrane strip.
- 6. Prepare different secondary antibody solutions within the recommended antibody range. Incubate each membrane strip in antibody solution for 1 hour at room temperature with agitation.
- 7. Briefly rinse the membrane with two changes of wash buffer. Wash the membrane by suspending it in wash buffer and agitate for 5 minutes in room temperature. Replace wash buffer at least 4 to 6 times.
- 8. Detect using Amersham ECL start Western blotting detection reagent detailed in *Detection, on page 18* of the protocol. The antibody dilution, which gives the best signal with minimum background should be selected.

9. Troubleshooting guide

Problems	Possible causes / remedies
Problems No signal	 Non-detectable amounts of target protein. Primary antibody is not binding to target protein, which may be due to bad quality and/or unspecific primary antibody. Incorrect species of secondary antibody has been used. PVDF membrane not pre-wetted in methanol/ ethanol. Check that transfer equipment is working properly and that the correct procedure has been followed. Check protein transfer by staining the membrane and/or gel. Confirm transfer efficiency by using pre-stained Amersham ECL Rainbow marker. Some proteins may be affected by the treatments
	 required for electrophoresis. Detection reagents do not function properly. To test the detection reagent activity, in a darkroom prepare 1 to 2 ml of detection reagent working solution in a clear test tube. Add 1 µl of undiluted HRP-conjugated antibody solution. The solution should immediately emit a visible blue light that fades during the next several minutes. Incorrect storage of Amersham ECL start Western blotting detection reagent may cause a loss of signal. Bacterial growth inhibit the reagent.

Problems	Possible causes / remedies
Weak signal	 Transfer efficiency may have been poor. Insufficient protein was loaded on to the gel. The concentration of primary and secondary antibodies could be too low; optimization is required. Bad quality and/or unspecific primary antibody. Exposure time may have been too short. Too much protein was loaded on to the gel.
Excessive, diffuse signal	 Too much protein was loaded on to the gel. The concentrations of primary and secondary antibodies could be too high; optimization is required.
White (negative) bands on the film	 Negative bands generally occur when protein target is in excess and antibody concentrations are too high. The effect is caused by substrate depletion. Load less amount of protein. Dilute both primary and secondary antibody further.
Uneven, spotted background	 Areas of the blot may have dried during some of the incubations. Incorrect handling can lead to contamination on the blots and/or membrane damage which may cause non-specific signal. The blocking agent is not completely dissolved in the buffer. Insufficient washing. Add additional washing steps.

Problems	Possible causes / remedies
High backgrounds	Too high concentrations of primary and secondary antibodies; optimization is required.
	 Insufficient washing. Use sufficient amount of wash buffer and add additional washing steps.
	 Transfer and incubation buffers may have become contaminated and require replacing. Always use fresh solutions
	Insufficient blocking.
	 The blocking agent used was not freshly prepared or was too dilute or was incompatible with the application.
	 The level of Tween used in the blocking agent was not sufficient for the application performed.
	• The membrane was allowed to dry during some of the incubations.
	• Poor gel quality.
	• Unspecific and bad quality of antibodies.
	• The film detection of the signal was allowed to over expose.
	 The level of signal is so high that the film has become completely overloaded.
	Non compatible products.

10. Related products

This chapter presents a subset of related products. For more information, refer to <u>www.gelifesciences.com/ecl</u>

10.1 Sample preparation

Product	Quantity	Code no.
SDS-PAGE Clean-up kit	50 samples	80-6484-70
Mammalian Protein Extraction Buffer	1 × 500 ml	28-9412-79
2-D Quant Kit	500 assays	80-6483-56

10.2 Molecular weight markers

Product	Quantity	Code no.
Amersham ECL Rainbow Markers	250 µl	RPN755E
Amersham ECL Rainbow Markers	250 µl	RPN756E
Amersham ECL Rainbow Markers	250 µl	RPN800E
Amersham ECL DualVue Western Blotting Markers	1 pack (25 loadings)	RPN810
Amersham ECL Plex™ Fluorescent Rainbow Markers	120 µl	RPN850E
Amersham ECL Plex Fluorescent Rainbow Markers	500 µl	RPN851E

10.3 Gel electrophoresis equipment

Product	Quantity	Code no.
Gel electrophoresis		
Amersham ECL Gel Box	1	28-9906-08
Amersham ECL Gel 10%, 10 wells	10	28-9898-04
Amersham ECL Gel 12%, 10 wells	10	28-9898-05
Amersham ECL Gel 4–12%, 10 wells	10	28-9898-06
Amersham ECL Gel 8–16%, 10 wells	10	28-9898-07
Amersham ECL Gel 4–20%, 10 wells	10	28-9901-54
Amersham ECL Gel 10%, 15 wells	10	28-9901-55
Amersham ECL Gel 12%, 15 wells	10	28-9901-56
Amersham ECL Gel 4–12%, 15 wells	10	28-9901-57
Amersham ECL Gel 8–16%, 15 wells	10	28-9901-58
Amersham ECL Gel 4–20%, 15 wells	10	28-9901-59

Product	Quantity	Code no.
Power Supplies		
EPS 301 Power Supply	1	18-1130-01

10.4 Transfer equipment

Product	Quantity	Code no.
Tank transfer units		
TE 22 Mini Tank Transfer Unit	1	80-6204-26
ECL Semidry Blotters		
TE 70 Semi-Dry Transfer Unit, 14 × 16 cm	1	80-6210-34
TE 70 PWR Semi-Dry Transfer Unit, 14 × 16 cm ¹	1	11-0013-41
TE 77 Semi-Dry Transfer Unit, 21 × 26 cm	1	80-6211-86
TE 77 PWR Semi-Dry Transfer Unit, 21 × 26 cm	1	11-0013-42

10.5 Blotting papers and membranes

Product	Quantity	Code no.
Blotting paper		
Hybond blotting paper (20 × 20 cm)	100 sheets	RPN6101M
3MM Chr 20 × 20 cm	100 sheets	3030-861
Nitrocellulose Membrar	nes	
Amersham Protran 0.1 NC (30 cm × 4 m)	1 roll	10600000
Amersham Protran 0.2 NC (30 cm × 4 m)	1 roll	10600001
Amersham Protran 0.45 NC (30 cm × 4 m)	1 roll	10600002
Amersham Protran Premium 0.2 NC (30 cm × 4 m)	1 roll	10600004
Amersham Protran Premium 0.45 NC (30 cm × 4 m)	1 roll	10600003
PVDF Membranes		
Amersham Hybond P 0.2 PVDF (26 cm × 4 m)	1 roll	10600021
Amersham Hybond P 0.45 PVDF (30 cm × 4 m)	1 roll	10600023

10.6 Blocking agents

Product	Quantity	Code no.
Amersham ECL Blocking Agent	40 g	RPN2125
Amersham ECL Prime Blocking Agent	40 g	RPN418

10.7 Amersham ECL HRP-linked secondary antibodies

Product	Quantity	Code no.
Amersham ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	1 ml	NA931-1ML
Amersham ECL Rabbit IgG, HRP-Linked Whole Ab (from donkey)	1 ml	NA934-1ML
Amersham ECL Mouse IgG, HRP-Linked F(ab)2 fragment (from sheep)	1 ml	NA9310-1ML
Amersham ECL Rabbit IgG, HRP-Linked F(ab)2 fragment (from donkey)	1 ml	NA9340-1ML

10.8 Detection reagents

Product	Quantity	Code no.
Amersham ECL start Western Blotting Detection Reagent	for 2000 cm ²	RPN3243
Amersham ECL start Western Blotting Detection Reagent	for 4000 cm ²	RPN3244
Amersham ECL start Western Blotting Detection Reagent	for 200 cm ²	RPN3245
Amersham ECL Select Western Blotting Detection Reagent	for 1000 cm ²	RPN2235
Amersham ECL Prime Western Blotting Detection Reagent	for 1000 cm ²	RPN2232
Amersham ECL Western Blotting Detection Reagents	for 1000 cm ²	RPN2109
Amersham ECL Western Blotting Detection Reagents	for 4000 cm ²	RPN2106
Amersham ECL Western Blotting Detection Reagents	for 6000 cm ²	RPN2134

10.9 X-ray films

Product	Quantity	Code no.
Amersham Hyperfilm ECL (5 × 7 inches)	50 sheets	28-9068-35
Amersham Hyperfilm ECL (18 × 24 cm)	50 sheets	28-9068-36
Amersham Hyperfilm ECL (8 × 10 inches)	50 sheets	28-9068-38

10.10 Imaging systems

Product	Quantity	Code no.
ImageQuant LAS 500	1	29-0050-63
Amersham Imager 600	1	29-0834-61

10.11 Software and accessories

Product	Quantity	Code no.
CD and getting started:		
ImageQuant TL and IQTL SecurITy Software package (with Getting Started Guide)		28-9380-94
Licenses for ImageQuant TL only:		
ImageQuant TL, single user license		28-9236-62

Product	Quantity	Code no.
Licenses for ImageQuant TL and IQTL SecurITy:		
ImageQuant TL and ImageQuant TL SecurITy	1-user	28-9332-73

GE Healthcare offices:

GE Healthcare Bio-Sciences AB Biörkaatan 30, 751,84 Uppsala Sweden GE Healthcare Europe GmbH Munzinger Strasse 5 D-79111 Freiburg Germany GE Healthcare Bio-Sciences Corp. 800 Centennial Avenue P.O. Box 1327 Piscataway. NJ 08855-1327 USA GE Healthcare Japan Corporation Sanken Blda. 3-25-1 Hyakunincho Shinjuku-ku Tokvo 169-0073 Japan

For your local office contact information, visit www.gelifesciences.com/contact

GE Healthcare UK Limited Amersham Place Little Chalfont, Buckinghamshire, HP7 9NA, UK

http://www.gelifesciences.com



29-1171-61 AC 11-2014