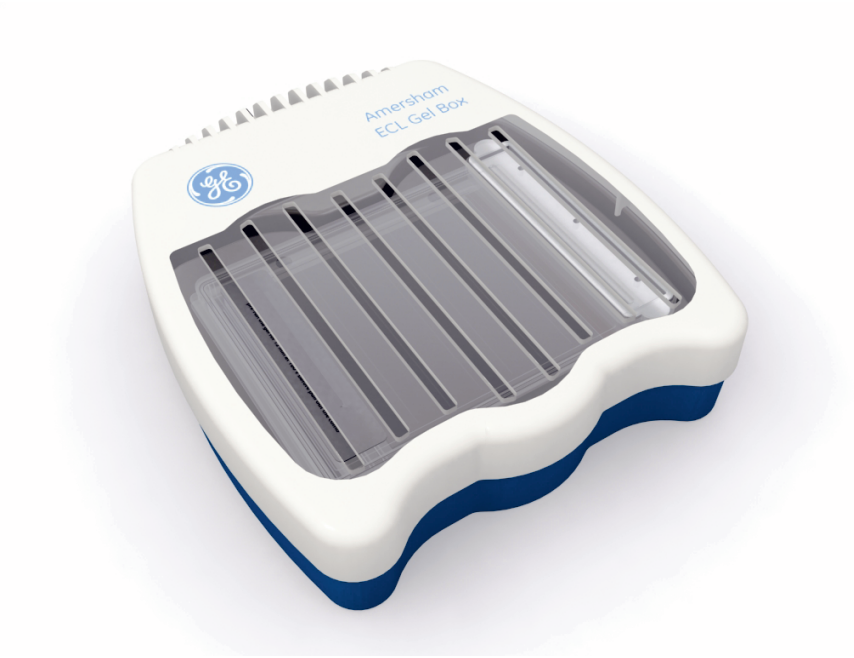
GE Healthcare
Life Sciences

Amersham™ ECL™ Gel Box

User Manual



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1 Introduction

General information

Amersham ECL Gel Box together with Amersham ECL Gel constitute a ready-to-use mini-gel system for high quality separation of proteins. Amersham ECL Gel is compatible with Tris-Glycine buffer and contains no SDS, making it ideal for protein analysis under both native and denaturing conditions. Amersham ECL Gel is compatible with standard protein detection protocols, and has an extended shelf-life for increased convenience.

Scope of this document

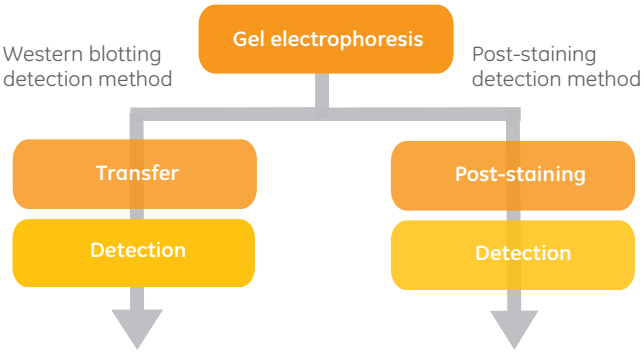
These instructions provide information on how to perform polyacrylamide gel electrophoresis (PAGE) using Amersham ECL Gel Box and Amersham ECL Gel. Additionally, protocols for post-staining and transfer are provided.

In this chapter

This chapter contains the following sections:

1.1 Protein gel electrophoresis overview

Below is a flowchart of protein analysis including separation of proteins using PAGE followed by either Western blotting or post-staining detection methods.



1.2 Important user information

Intended use

Amersham ECL Gel Box and Amersham ECL Gel are intended for PAGE.

Amersham ECL Gel Box and Amersham ECL Gel are intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of Amersham ECL Gel Box. 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.

1.3 Regulatory information

Introduction

This section describes the directives and standards that are fulfilled by Amersham ECL Gel Box.

CE Conformity

This product complies with the European directives listed in the table below, by fulfilling the corresponding harmonized standards. A copy of the Declaration of Conformity is available on request.

Directive	Title
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	Electromagnetic Compatibility (EMC) Directive

CE Marking

CE

The CE marking and the corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE marked GE Healthcare instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation.

International standards

This product fulfills the requirements of the following standards:

Standard	Description	Notes
EN 61010-1, IEC 61010-1, UL 61010-1, CAN/CSA-C22.2 No.61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use.	Harmonized with 2006/95/EC
EN 61010-031, IEC 61010-031	Safety requirements for hand-held probe assemblies for electrical measurement and test.	Harmonized with 2006/95/EC
EN 61326-1	EMC emissions and immunity requirements for electrical equipment for measurement, control and laboratory use.	Harmonized with 2004/108/EC

Regulatory compliance of connected equipment

Any equipment connected to Amersham ECL Gel Box should meet the safety requirements of EN 61010-1/IEC 61010-1, or other relevant harmonized standards. Within EU, connected equipment must be CE marked.

2 Safety

About this chapter

This chapter presents safety precautions for Amersham ECL Gel Box. Labels on the instrument and information regarding recycling are also included in this chapter.

In this chapter

This chapter contains the following sections:

2.1 Safety precautions

General precautions

WARNING

Do not use Amersham ECL Gel Box in any other way than described in the Amersham ECL Gel Box Instructions.

CAUTION

Operation of Amersham ECL Gel Box should be performed by properly trained personnel only.

CAUTION

Do not use any accessories not supplied or recommended by GE Healthcare.

Installation

WARNING

Access to power switch and power cord. Do not block access to the Power switch on the Power supply and the Power cord. The Power switch must always be easy to access. The Power cord must always be easy to disconnect.

CAUTION

During electrophoresis, very low quantities of gases are produced at the electrodes. Make sure that Amersham ECL Gel Box is run in a well ventilated area.

CAUTION

Amersham ECL Gel Box is designed for indoor use only.

Operation

WARNING

High voltage. Before use, check that Amersham ECL Gel Box is completely dry on the outside before connecting it to a power supply. Wipe dry with a cloth.

WARNING

Do not use Amersham ECL Gel Box if it is not working properly, nor if it has suffered any damage, for example:

- damage caused by dropping the equipment
- damage by spilling liquid onto it.

WARNING

High voltage. Do not connect the high voltage cable to an external power supply if it is not working properly, nor if it has suffered any damage, for example damage to its plug or cable.

WARNING

Electrical shock hazard. Always switch off the power supply and disconnect Amersham ECL Gel Box from the power supply before removing the safety lid.

WARNING

Do not operate Amersham ECL Gel Box without a full-functioning safety lid in place.

WARNING

Do not exceed the maximum operating voltage of 200 V ⎓.

WARNING

Do not operate the Amersham ECL Gel Box inside a metal tray.

WARNING

Never operate/run the Amersham ECL Gel Box without both high voltage plugs connected to the external power supply.

WARNING

High voltage. Do not overfill Amersham ECL Gel Box with running buffer. Maximum volume is 90 ml per tank.

WARNING

Do not operate Amersham ECL Gel Box close to strong magnetic fields.

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.

CAUTION

Do not move Amersham ECL Gel Box during operation.

NOTICE

To prevent equipment damage, only operate Amersham ECL Gel Box in a horizontal position.

Maintenance

WARNING

Electrical shock hazard. Always switch off the Power supply and disconnect Amersham ECL Gel Box from the Power supply before maintenance and cleaning.

WARNING

Cleaning. Do not autoclave, bake or microwave Amersham ECL Gel Box.

WARNING

Cleaning. Do not wash Amersham ECL Gel Box in a mechanical washer.

WARNING

Cleaning. Do not use abrasive creams or scourers.

NOTICE

Cleaning. Clean the instrument with distilled water and wipe dry with a soft damp tissue. Let the instrument dry completely before use.

Materials Safety Data Sheet

It is recommended to read the Materials Safety Data Sheet (MSDS) before using Amersham ECL Gel.

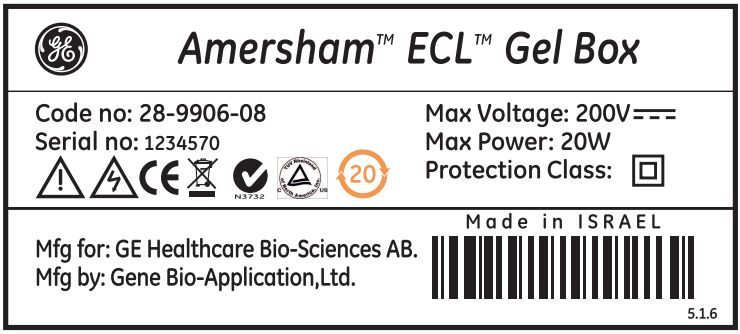
2.2 Labels

Introduction

This section describes the safety labels and labels concerning hazardous substances that are attached to the Amersham ECL Gel Box instrument.

Labels on Amersham ECL Gel Box

The illustration below shows an example of the label that is attached to the bottom of the Amersham ECL Gel Box.



Safety symbols

The following safety symbols are used in the label:

Label	Meaning
	**Warning!** Read the user documentation before using the instrument. Do not open any covers or replace parts unless specifically stated in the user documentation.

Label	Meaning
	**Warning! High Voltage**. Always make sure that the instrument is disconnected from electric power before opening the lid or disconnecting any electric equipment.
	The instrument complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand. N3732.
	The instrument complies with applicable European directives.
	The instrument is listed according to TÜV Rheinland.
	The instrument is protected throughout by double insulation.

Labels concerning hazardous substances

The following symbols on the labels concern hazardous substances:

Label	Meaning
	This symbol indicates that electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard *SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronic Information Product*.

2.3 Recycling information

Decontamination

Amersham ECL Gel Box shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking Amersham ECL Gel Box out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

2.4 Declaration of Hazardous Substances (DoHS)

Introduction

The following product pollution control information is provided according to SJ/T11364-2006 Marking for Control of Pollution caused by Electronic Information Products.

根据SJ/T11364-2006《电子信息产品污染控制标识要求》特提供如下有关污染 控制方面的信息

Symbols used in pollution control label

电子信息产品污染控制标志说明

Label	Meaning
	This symbol indicates the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronic Information Products. The number in the symbol is the Environment-friendly Use Period (EFUP), which indicates the period during which the toxic or hazardous substances or elements contained in electronic information products will not leak or mutate under normal operating conditions so that the use of such electronic information products will not result in any severe environmental pollution, any bodily injury or damage to any assets. The unit of the period is “Year”. In order to maintain the declared EFUP, the product shall be operated normally according to the instructions and environmental conditions as defined in the product manual, and periodic maintenance schedules specified in Product Maintenance Procedures shall be followed strictly.

Label	Meaning
	Consumables or certain parts may have their own label with an EFUP value less than the product. Periodic replacement of those consumables or parts to maintain the declared EFUP shall be done in accordance with the Product Maintenance Procedures. This product must not be disposed of as unsorted municipal waste, and must be collected separately and handled properly after decommissioning.
	该标志表明本产品含有超过SJ/T11363-2006《电子信息产品中有毒有害物质的限量要求》中限量的有毒有害物质。标志中的数字为本产品的环保使用期，表明本 产品在正常使用的条件下，有毒有害物质不会发生外泄或突变，用户使用本产品 不会对环境造成严重污染或对其人身、财产造成严重损害的期限。单位为年。 为保证所申明的环保使用期限，应按产品手册中所规定的环境条件和方法进行正 常使用，并严格遵守产品维修手册中规定的期维修和保养要求。 产品中的消耗件和某些零部件可能有其单独的环保使用期限标志，并且其环保使 用期限有可能比整个产品本身的环保使用期限短。应到期按产品维修程序更换那 些消耗件和零部件，以保证所申明的整个产品的环保使用期限。 本产品在使用寿命结束时不可作为普通生活垃圾处理，应被单独收集妥善处理

List of hazardous substances and their concentrations

产品中有毒有害物质或元素的名称及含量

Indication for each major part if substance exceeds limit

Value	Meaning
0	Indicates that this toxic or hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement in SJ/T11363-2006. 表示该有毒有害物质在该部件所有均质材料中的含量均在SJ/T11363-2006 标准规定的限量要 求以下
X	Indicates that this toxic or hazardous substance contained in at least one of the homogeneous materials used for this part is above the limit requirement in SJ/T11363-2006. • Data listed in the table represents best information available at the time of publication • Applications of hazardous substances in this medical device are required to achieve its intended clinical uses, and/or to provide better protection to human beings and/or to environment, due to lack of reasonably (economically or technically) available substitutes. 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出SJ/T11363-2006 标准规定的 限量要求 • 此表所列数据为发布时所能获得的最佳信息 • 由于缺少经济上或技术上合理可行的替代物质或方案，此医疗设备运用以上一些有毒有害物质来实现设备的预期临床功能，或给人员或环境提供更好的保护效果。

List of hazardous substances

Component name 部件名称	Hazardous substance 有毒有害物质或元素					
	Pb 铅	Hg 汞	Cd 镉	Cr6+ 六价铬	PBB 多溴联苯	PBDE 多溴二苯醚
Amersham ECL Gel Box, 28-9902-52 [1]	0	0	0	0	0	0

1 The product has not been tested as per the Chinese standard *SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronic Information Product.*

3 System description

About this chapter

This chapter describes the features of Amersham ECL Gel Box and Amersham ECL Gel.

In this chapter

This chapter contains the following sections:

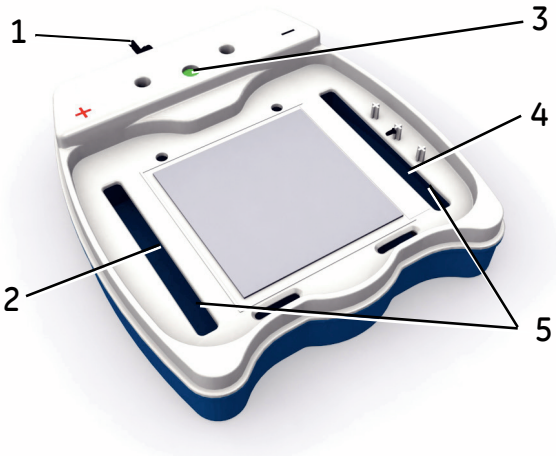
3.1 Amersham ECL Gel Box

Introduction

This section describes the features of Amersham ECL Gel Box.

Overview

The illustration below shows the main parts of Amersham ECL Gel Box.



Part	Description	Part	Description
1	Power cord (to external power supply)	4	Cathode (located underneath the plastic case)
2	Anode (located underneath the plastic case)	5	Buffer tanks (for running buffer)
3	Indicator light		

Gel Box specifications

Parameter	Value
Dimensions: Gel Box Gel cassette	 167 x 148 x 43.5 mm (W x H x D) 116.8 x 84 x 15.6 mm (W x H x D)
Maximum voltage	200 V ⎓
Operating temperature	4°C to 40°C
Recommended power supply	EPS 301
Maximum power	20 W
Maximum current	100 mA
Degree of protection	IPX5

ECL Gel cassette

The image below shows the Amersham ECL Gel cassette.

Part	Description	Part	Description
1	Comb	3	Cassette
2	Well container		

Power supply

Amersham ECL Gel Box is powered by an external power supply (EPS 301). See *Power supplies, on page 63* for more information. If other power supply with a Ø 4 mm, deep socket (>6 mm internal distance between surface and socket) is used, it is recommended to use the adapter delivered with Amersham ECL Gel Box. See *Connecting other power supplies, on page 35* for more information.

Safety lid

Amersham ECL Gel Box is equipped with a safety lid, which shall be placed on top of the base unit before a power supply is connected.

The safety lid has two magnets which will activate current only when their magnetic fields are in contact with the electrodes inside the base unit of Amersham ECL Gel Box. This means that Amersham ECL Gel Box is only powered when the safety lid is in the correct position. Removing the safety lid will switch off the high voltage to the electrodes.

WARNING

Do not operate Amersham ECL Gel Box without the safety lid.

3.2 Amersham ECL Gel

Introduction

This section describes the features of Amersham ECL Gel.

Design

Amersham ECL Gel is designed for PAGE using Amersham ECL Gel Box.

Gel concentrations

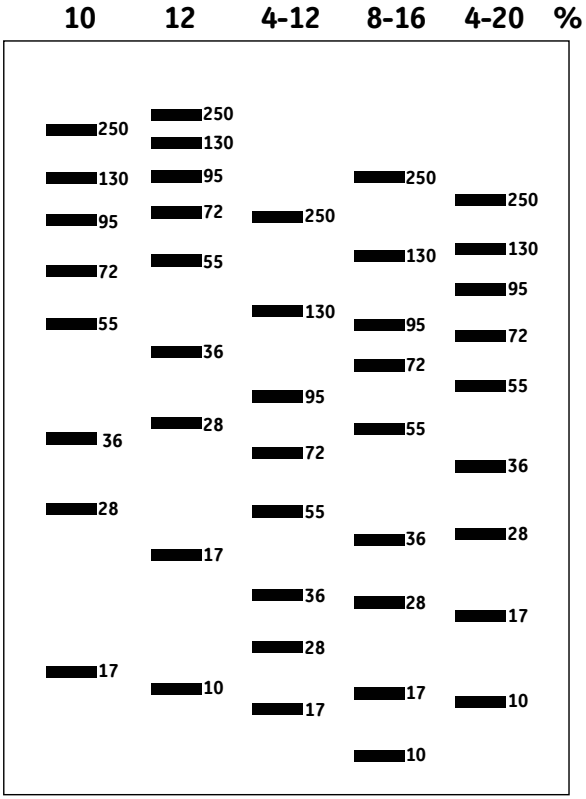
Amersham ECL Gels are available as homogenous and gradient gels in the following concentrations:

- 10%
- 12%
- 4-12%
- 8-16%
- 4-20%

For information regarding separation ranges for each Amersham ECL Gel, see *Separation range, on page 31*.

Separation range

The following image shows the separation pattern of proteins at different molecular weight for each Amersham ECL Gel.



Gel specifications

Parameter	Value
Gel matrix	Acrylamide/bis-acrylamide
Gel concentration	%C=2.6% (w/w) %T=Total concentration (w/v) of acrylamide + bisacrylamide is given as percentage in the respective Amersham ECL Gel product name.
Gel thickness	1.4 mm
Gel size	7.5 cm x 8.0 cm (W x H)
Sample well configuration	2, 10, and 15 well gels are available.
Sample well volume 2 wells 10 wells 15 wells	 100 µl 35 µl 20 µl
Stacking gel	4%
Buffer system in gel	Tris-HCl
Running buffer: • Denatured conditions • Native conditions	 • 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3 • 25 mM Tris, 192 mM glycine, pH 8.3
Sample buffer	e.g. Tris-HCl-SDS or any other buffer suitable for the application
Shelf life	12 months from date of manufacture

Buffer solutions

Solution	Preparations
2× SDS Sample buffer	1 Mix 2.5 ml of 0.5 M Tris-HCl, pH 6.8, 4.0 ml of 10% (w/v) SDS, 2.0 ml of glycerol, 2.0 mg of bromphenol blue and 310 mg of DTT. 2 Make up to 10 ml with distilled water.
Amersham ECL Gel Running buffer, 1×	Amersham ECL Gel Running Buffer, 10× contains 250 mM Tris HCl, 1.92 M glycine, 1% SDS Prepare 1× buffer for denatured conditions by adding distilled water in a 1:10 dilution.

4 Installation

In this chapter

This chapter describes how to connect Amersham ECL Gel Box to a power supply.

Precautions

WARNING

Access to power switch and power cord. Do not block access to the Power switch on the Power supply and the Power cord. The Power switch must always be easy to access. The Power cord must always be easy to disconnect.

CAUTION

During electrophoresis, very low quantities of gases are produced at the electrodes. Make sure that Amersham ECL Gel Box is run in a well ventilated area.

CAUTION

Amersham ECL Gel Box is designed for indoor use only.

Connecting GE Healthcare power supplies

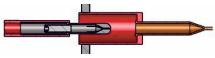
Connect Amersham ECL Gel Box to the power supply according to the instructions supplied with the power supply.

Connecting other power supplies

The power supply must have a rigid insulating sleeve output connector that fulfills the requirements for 200 V ⎓ (DC), (for example EPS 301). If a power supply with Ø 4 mm, deep socket (>6 mm internal distance between surface and socket) is used, it is recommended to use the adapter delivered with Amersham ECL Gel Box. For mounting instructions, see *Mounting the adapter, on page 37*.

Note: *Amersham ECL Gel Box is delivered with two adapters, identical apart from color, one black (-) and one red (+) (shown above).*

Power supply compatibility guide for Amersham™ ECL™ Gel Box

Power supply type	Adapter	Example
Equipped with Ø 4 mm rigid insulation	Not needed	GEHC EPS 301
Without Ø 4 mm rigid insulation; inner distance >6 mm	Adapter supplied with Amersham ECL Gel Box 	
High-voltage power supplies with Ø 2 mm rigid insulation	18-1129-59 Adapter set (4 mm/2 mm) for use with 4 mm HV leads to 2 mm power supply output socket 	
GEHC EPS power supply with built-in sudden load change detection	Not compatible	EPS 601, EPS 1001, EPS 3501XL

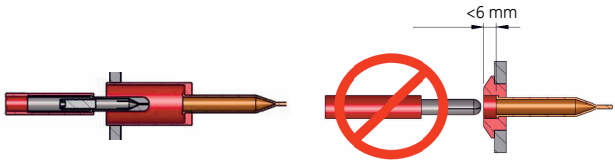
Mounting the adapter

This instruction describes how to mount the adapter delivered with Amersham ECL Gel Box

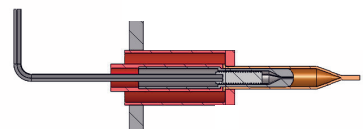
Step	Action
1	Turn off the power supply and remove the electrical contact from the power supply outlet.
2	Insert the adapter in the power supply output socket.

WARNING

Do not use the adapter in Ø 4 mm socket with <6 mm internal distance between surface and socket.



3	Tighten the set screw using the 1.5 mm Hex wrench provided with the Amersham ECL Gel Box, to lock the expandable plug of the adapter into the socket.



4	Make sure that the adapter is locked into the socket.

5 Protocols

About this chapter

This chapter presents protocols for PAGE using Amersham ECL Gel Box and Amersham ECL Gel. Additionally, protocols for post-staining and transfer are provided.

In this chapter

This chapter contains the following sections:

5.1 Electrophoresis protocol

In this section

This section describes how to perform PAGE using Amersham ECL Gel Box and Amersham ECL Gel.

Precautions

WARNING

Do not use Amersham ECL Gel Box if it is not working properly, nor if it has suffered any damage, for example:

- damage caused by dropping the equipment
- damage by spilling liquid onto it.

WARNING

Do not connect the high voltage cable to an external power supply if it is not working properly, nor if it has suffered any damage, for example damage to its plug or cable.

Preparations before a run

Step	Action
1	Prepare 1× running buffer by diluting 19 ml of Amersham ECL Gel Running Buffer, 10× in 171 ml water. 190 ml buffer is sufficient for one electrophoresis gel.
2	Add 90 ml of 1× running buffer to each tank of Amersham ECL Gel Box.
3	Cut open the gel package and gently remove Amersham ECL Gel from the package.
4	Rinse the gel cassette with distilled water. Peel off the tapes from the two legs of the cassette.
5	Place Amersham ECL Gel in Amersham ECL Gel Box so that the well side of the cassette faces toward the cathode (-) and the other cassette leg faces toward the anode (+).
6	Place the safety lid on top of Amersham ECL Gel Box.
7	Connect Amersham ECL Gel Box to the power supply (EPS 301) and pre-run the gel for 12 minutes at 160 V.

WARNING

Do not exceed the maximum operating voltage of 200 V ⎓.

Note:

Expected start current approx. 45 mA.

8	Once the pre-run is finished, switch off the power.
9	Remove the safety lid.

Step	Action
10	Wiggle the comb back and forth, and bring it straight up from the cassette to make the wells available for sample loading.

Note:

Small gel pieces can be detached from the well container. This will not affect gel performance.

Note:

Do not discard the comb.

11	Add 6 ml of 1× running buffer to the well container.

Sample loading

To load the samples, follow the instructions:

Step	Action
1	Prepare the samples by adding sample and 2× sample buffer in a 1:1 mixture. Example: for 10 µl sample, add 10 µl 2× sample buffer. ***Note:*** *For native conditions: use a sample buffer without SDS and DTT.*
2	Heat the samples at 95°C for 5 minutes.
3	Spin down the samples quickly in a microcentrifuge and load the samples directly into the wells in the gel. ***Note:*** *A maximum of 0.5 µg/band per well of sample can be loaded. Overloading may cause smearing and distortion.* ***Note:*** *To ensure uniform mobility, load an equal volume of 1× SDS sample buffer into any unused well.*
4	Place the safety lid on top of Amersham ECL Gel Box.

Running conditions

Run the gel according to the recommendations below.

Parameter	Value
Voltage	160 V ⎓
Expected current	Start: approx. 45 mA End: 23 to 33 mA

Parameter	Value
Run time	60 minutes

WARNING

Do not exceed the maximum operating voltage of 200 V ⎓.

5.2 Removing the gel from the cassette

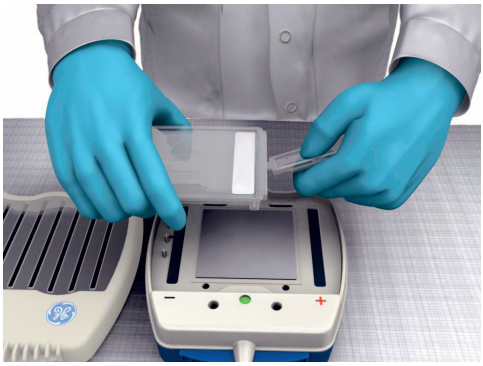
In this section

This section describes how to remove the gel from the Amersham ECL Gel Box cassette after an electrophoresis run.

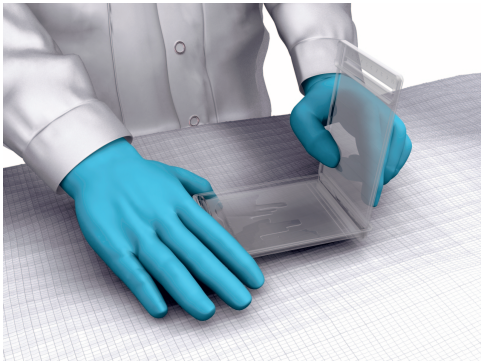
Removing the gel from the cassette

To remove the gel, do the following:

Step	Action
1	Once the run is completed, shut off the power, disconnect the electrodes, remove the safety lid and finally, remove the gel cassette from Amersham ECL Gel Box.
2	Open the cassette by inserting the edge of the comb in the slot opposite the sample wells, and twist.



3	Remove the top plate from the gel cassette and allow the gel to sit on the bottom plate.

Step	Action
4	Cut the stacking gel with the end of the top plate approximately 2 cm downstream of the wells. Repeat the procedure on the other side of the gel to remove the front. 
5	Hold the gel cassette bottom plate over a container with suitable buffer, with the gel facing downwards. Gently push a tweezer between the gel and the cassette bottom plate until the gel is removed from the bottom plate.

5.3 Post-staining of the gel

In this section

All standard staining procedures, including Coomassie staining, Silver staining and Deep Purple™ staining, may be used with Amersham ECL Gel. For staining solutions recommended by GE Healthcare, see *Gel staining reagents, on page 64*.

Coomassie staining

Introduction

This section presents a Coomassie staining protocol recommended by GE Healthcare.

Coomassie staining solutions

250 ml of each solution is used in each step.

Solution	Preparation
Fixing solution	1 Mix 400 ml of ethanol and 100 ml of acetic acid with distilled water. 2 Make up to 1.0 l with distilled water
Staining solution	1 Mix 1 tablet of PhastGel™ Blue R-350 with destaining solution. 2 Make up to 400 ml with destaining solution. Heat to 60°C, stirring constantly, and filter before use.
Washing solution	Distilled water
Destaining solution	1 Mix 250 ml ethanol and 80 ml acetic acid. 2 Make up to 1.0 l with distilled water.

Solution	Preparation
Preserving solution	1 Mix 25 ml of (87% v/v) glycerol with distilled water. 2 Make up to 250 ml with destaining solution.

Coomassie staining protocol

Step	Action
1	**Fixation:** Immediately after electrophoresis, immerse the gel in fixing solution for 30 minutes. This solution precipitates the proteins and allows the SDS to diffuse out of the gel.
2	**Staining:** Discard the fixing solution. Stain the gel for 10 minutes in staining solution. Cover the staining dish.
3	**Washing:** Rinse the gel once in distilled water.
4	**Destaining:** Destain the gel by changing the destaining solution several times until the stained protein bands are clearly visible against the clear background.
5	**Preserving:** Soak the destained gel in preserving solution for 30 minutes.

Deep purple staining

Introduction

This section presents a Deep Purple staining protocol recommended by GE Healthcare. For more information refer to RPN6305 Deep Purple Total Protein Stain.

Deep Purple staining solutions

Solution	Preparation
Fixating/ Acidifying solution	1 Mix 150 ml ethanol and 10 g citric acid (approx. pH 2.3). 2 Make up to 1.0 l with distilled water. ***Note:*** *Fixing solution can be stored at room temperature for up to six months.*
Working staining solution	1 Disolve 6.2 g boric acid in 800 ml distilled water. Adjust to pH 10.5 with NaOH. 2 Make up to 1.0 l with distilled water. ***Note:*** *Boric acid / sodium hydroxide buffer can be stored at room temperature for up to six months.* ***Note:*** *Staining solution should be made fresh just before use by adding 1 part Deep Purple to 200 parts borate buffer for gels. Prior to diluting with borate buffer, ensure that the Deep Purple concentrate is at room temperature.*

Solution	Preparation
Washing solution	1 Mix 150 ml ethanol with distilled water. 2 Make up to 1.0 l with distilled water. ***Note:*** *Washing solution can be stored at room temperature for up to six months.*
Storing solution	1 Add 10 g citric acid to 500 ml distilled water and adjust to pH 2.3. 2 Make up to 1.0 l with distilled water. ***Note:*** *Gels should be stored in storing solution at 4°C in a dark location or away from light.*

Deep Purple staining protocols

Step	Action
1	**Fixing:** Fix gels from 90 minutes to overnight in fixing solution.
2	**Staining:** Stain for 90 minutes at room temperature covered from light using the prepared working staining solution. Prior to diluting with borate buffer, ensure that the Deep Purple concentrate is at room temperature.
3	**Washing:** Wash for 90 minutes in washing solution.
4	**Acidifying:** Acidify gels for 45 minutes. Further washes in this solution can be performed if desired.
5	**Imaging:** Imaging is best performed using laser scanning fluorescence flat bed imaging systems, such as the GE Typhoon™ FLA laser scanners.

Step	Action
6	**Storing:** After imaging, the gels can be stored in the dark at -8°C for several weeks in storage solution (pH 2.3). ***Note:*** *This allows for further imaging at a later date, if required. For long term storage (up to 6 months) add Deep Purple stain in 1:200 dilution.*

Silver staining

Introduction

This section presents a Silver staining protocol recommended by GE Healthcare. Refer to Silver Staining Kit Protein 71-7177-00 for more information.

Silver staining solutions

Solutions	Preparations
Fixing solution	1 Mix 75 ml ethanol and 25 ml glacial acetic acid. 2 Make up to 250 ml with distilled water.
Sensitizing solution	1 Mix 75 ml ethanol, 10 ml sodium thiosulfate (5% w/v) and 1 packet of sodium acetate (17 g). 2 Make up to to 250 ml with distilled water. **Before use:** Add 1.25 ml glutaraldehyde (25% w/v).
Staining solution	1 Mix 25 ml of silver nitrate solution (2.5% w/v) with distilled water. 2 Make up to 250 ml with distilled water. **Before use:** Add 0.1 ml formaldehyde (37% w/v).

Solutions	Preparations
Developing solution	• Mix 1 packet of sodium carbonate (6.25 g) and distilled water. • Make up to 250 ml with distilled water. ***Note:*** *Stir vigorously to dissolve the sodium carbonate* **Before use:** Add 0.2 ml of formaldehyde (37% w/v).
Stop solution	1 Mix EDTA-Na_2•$2H_2O$ (3.65 g) with distilled water. 2 Make up to 250 ml with distilled water.
Washing solution	Distilled water
Preserving solution	1 Mix 75 ml of ethanol and 11.5 ml of glycerol (87% w/w). 2 Make up to 250 ml with distilled water.

Silver staining protocol

Step	Action
1	**Fixation:** Soak the gel in fixing solution for 120 minutes (or overnight).
2	**Sensitizing:** Discard the solution. Add sensitizing solution and gently shake for at least 120 minutes.
3	**Washing:** Remove the sensitizing solution. Add distilled water and wash five times for 15 minutes each time. Leave in washing solution over night.
4	**Silver reaction:** Add Staining solution and gently shake for 120 minutes.

Step	Action
5	**Washing:** Remove the Staining solution. Rinse three times in distilled water for one minute each time.
6	**Developing:** Add developing solution and gently shake for 3 minutes. Transfer the gel to stopping solution when the bands/spots have reached desired intensity. ***Note:*** *If the background and and the bands are too strong, use a cold transfer buffer and/or transfer the gel to stopping solution before desired density has been reached.*
7	**Stopping:** Gently shake the gel in stopping solution for 120 minutes.
8	**Preserving:** Add preserving solution and shake gently for 120 minutes.

5.4 Transfer protocol

In this section

This section describes how to transfer the proteins from the gel to a blotting membrane using tank transfer and semi-dry blotting.

Tank transfer

Transfer buffer

Tris-glycine buffer: 25 mM Tris, 192 mM glycine, 20% methanol. Prepare fresh.

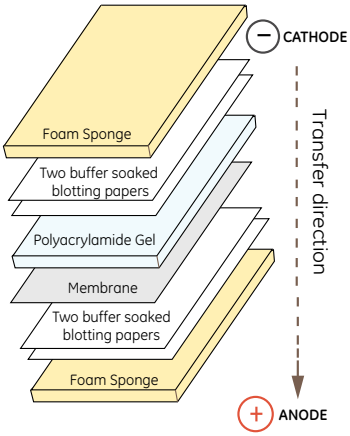
Note: *Use a pre-chilled transfer buffer.*

Preparing the membrane

Step	Action
1	Pre-wet the membranes: • NitroCellulose (NC) membrane: Pre-wet the membranes in distilled water for five minutes followed by 10 minutes in transfer buffer. • PVDF membrane: Pre-wet the PVDF membranes first in methanol for 20 seconds, then in distilled water for 5 minutes and finally, in cold transfer buffer for at least 10 minutes. ***Note:*** *Use a pre-cut membrane provided by GE Healthcare. See Chapter 8 Related products, on page 62.*

Preparing the transfer tank

Step	Action
1	Fill the transfer unit with transfer buffer. For information on how to use the transfer unit, refer to the instructions provided by the manufacturer of the transfer unit.
2	Place the open transfer cassette with the anode side (+) down in a tray with transferbuffer and ensure that it is immersed in at least 3 cm of the transfer buffer.
3	Build the stack with the membrane closest to the anode side. Below is an illustration of the transfer sandwich.



4	Layer 1: Place a 3 mm thick foam sponge in the transfer cassette and press gently to remove any air bubbles.
5	Wet the blotting paper in transfer buffer.

Step	Action
6	Layer 2: Place the two pre-wetted blotting papers on the sponge and press gently to remove any air bubbles. ***Note:*** *Use a pipette or stirring rod to gently roll out any air bubbles trapped while assembling the stack.*
7	Layer 3: Place the pre-wetted membrane on layer 2 and remove all air-bubbles.
8	Equilibrate the gel in cold transfer buffer for 10 to 20 minutes.
9	Layer 4: Place the gel on the membrane and ensure there are no air bubbles.
10	Layer 5: Cover the gel with two pre-wetted sheets of blotting paper.
11	Layer 6: Finally place a sponge of 3 mm and again press gently to remove any air bubbles.
12	Close the cassette and press lightly to lock the tabs.
13	Place the cassette into the tank.
14	Run the transfer according to the recommendations from the transfer tank supplier.

Recommended running conditions for transfer

Parameter	Value
Voltage	25 V ⎓ (maximum 400 mA)
Temperature	4°C
Time	2.5 hours (with constant stirring)

Semi-dry transfer

Introduction

In semi-dry transfer, the gel is placed in direct contact with the membrane and several layers of filter papers soaked in transfer buffer are placed above and below the gel and the membrane.

- The filter papers, gel and membrane are sandwiched between two plates that form an anode and a cathode when an electric field is applied.
- The filter papers and the membrane should be carefully cut to be a few millimeters smaller than the length and the width of the gels. This ensures that the filter papers and membranes do not overlap the gel, forming a potential short cut for the current, leading to inefficient or uneven transfer of proteins.
- Ensure that no air bubbles form when applying the gel to the membrane. Bubbles will cause blank spots on the membrane where no protein transfer occurs.
- Prolonged semi-dry blotting may lead to overheating and gel drying due to buffer depletion. Semi-dry transfer works well for most proteins but may be less efficient for large proteins.

Protocol information

Please refer to 28-4025-91 for detailed instructions for use of Semi-dry blotters TE70 and TE77.

Post transfer

After transfer, continue with Western blotting detection or Post-staining detection. For information regarding GE Healthcare recommended products, see *Chapter 8 Related products, on page 62*.

6 Maintenance

Precautions

WARNING

Electrical shock hazard. Always switch off the power supply and disconnect Amersham ECL Gel Box from the power supply before maintenance and cleaning.

WARNING

Cleaning. Do not autoclave, bake or microwave Amersham ECL Gel Box.

WARNING

Cleaning. Do not wash Amersham ECL Gel Box in a mechanical washer.

WARNING

Cleaning. Do not use abrasive creams or scourers.

NOTICE

Cleaning. Clean the instrument with distilled water and wipe dry with a soft damp tissue. Let the instrument dry completely before use.

Cleaning

To clean Amersham ECL Gel Box, do as follows:

Step	Action
1	Discard the running buffer.
2	Rinse thoroughly with distilled water.
3	Let the instrument air dry, or, wipe with a soft damp tissue.
4	Keep power cables and connectors dry.

Storage

Store Amersham ECL Gel Box in a dry location at room temperature.

7 Troubleshooting

Problems	Possible causes	Remedies
Low or no current during the run	Interrupted circuit	• Add some running buffer to each tank of Amersham ECL Gel Box • Ensure that the tape is removed from the gel cassette • Ensure that the lid is in place
Streaking of proteins	• Sample overload • Poorly soluble or weakly charged particles (such as carbohydrates in sample) • High salt concentration in the sample • Contaminants such as membranes or DNA complexes in the sample	• Load the appropriate amount of protein • Centrifuge the samples • Change the pH of the sample buffer • Heat the sample together with SDS • Decrease the salt concentration of the sample solution using dialysis or gel filtration

Problems	Possible causes	Remedies
Bands difficult to distinguish	Incorrect gel selection, sample overloading	• Select a gel that separates in the desired molecular weight range • Reduce loaded protein amount • For proteins of similar molecular weight, a 2-D separation may be required
Sample spreading across the gel	• Excess salt in the sample • Too much protein applied to the gel	• Reduce salt by ultrafiltration • Optimize the amount of protein applied to the gel
Sample contains appreciable carbohydrate		• Remove the carbohydrate by enzymatic or chemical means
Sample contains lipoproteins		• Use a gradient gel • Try addition of a non-ionic detergent
Protein denaturation and band inversion	Excessive heating	• Start with cold buffer (<15°C)
Diffuse protein zones in the gel after staining	• SDS still present in the gel • Insufficient reduction of samples	• Wash the gels extensively (3 x 10 minutes) with ultrapure water and use 30% methanol to destain the gel • Use 10% TCA to fix the proteins in the gel. Add extra reducing agent or change the reducing agent.

Problems	Possible causes	Remedies
No green light, low current (<20 mA) at start.	Incomplete circuit	• Check all power connections • Check buffer level in tanks • Ensure that the gel cassette is in place • Ensure that there is no tape left on the gel cassette
Green light, but only approx. 35 mA at start.	Leak between tanks, with or without gel cassette	• Check that the tanks are not overfilled • Check if the unit is damaged and needs to be replaced
Green light, but approx. 65 mA at start	Leak between tanks with gel cassette	• Check that tanks are not overfilled • Check if the unit is damaged and needs to be replaced

8 Related products

In this chapter

This chapter presents a subset of related products. For more information, refer to www.gelifesciences.com.

Sample preparation

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

Amersham markers

Product	Quantity	Code no.
Low-Range Rainbow™ Molecular Weight Markers	250 µl	RPN755E
High-Range Rainbow Molecular Weight Markers	250 µl	RPN756E
Full-Range Rainbow Molecular Weight 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

Power supplies

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

Amersham ECL Gel

Product	Quantity	Code no.
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
Amersham ECL Gel 10%, 2 wells	10	28-9901-60
Amersham ECL Gel 12%, 2 wells	10	28-9901-61
Amersham ECL Gel 4-12%, 2 wells	10	28-9901-62
Amersham ECL Gel 8-16%, 2 wells	10	28-9901-63
Amersham ECL Gel 4-20%, 2 wells	10	28-9901-64

Product	Quantity	Code no.
Amersham ECL Gel 10%, 10 wells	2	28-9898-08
Amersham ECL Gel 12%, 10 wells	2	28-9898-09
Amersham ECL Gel 4-12%, 10 wells	2	28-9901-51
Amersham ECL Gel 8-16%, 10 wells	2	28-9901-52
Amersham ECL Gel 4-20%, 10 wells	2	28-9901-53

Electrophoresis buffers

Product	Quantity	Code no.
Amersham ECL Gel Running Buffer, 10× 250 mM Tris HCl, 1,92 M glycine, 1% SDS	2 x 125 ml	28-9902-52
Bromophenol Blue	10 g	17-1329-01

Gel staining reagents

Product	Quantity	Code no.
Deep Purple Total Protein Stain	5 ml 25 ml	RPN6305 RPN6306
PlusOne™ Silver Staining Kit, Protein	1	17-1150-01
PlusOne Coomassie Blue PhastGel R-350 Tablets	40 Tablets	17-0518-01

Transfer equipment

Product	Quantity	Code no.
TE 70 PWR Semi-Dry Transfer Unit, 14 x 16 cm	1	11-0013-41
TE 77 PWR Semi-Dry Transfer Unit, 21 x 26 cm	1	11-0013-42
Amersham ECL Multiprobe	1	11-0033-95
Amersham ECL Multiprobe XL	1	11-0033-96
TE 22 Mini Tank Transfer Unit	1	80-6204-26
TE 62 Transfer Unit	1	80-6209-58

Blotting membranes

Product	Quantity	Code no.
Blotting paper		
3 MM Chr 20 x 20 cm	100 sheets	3030-861
Blotting sandwich		
Protran™ BA83, 0.2 µm blotting sandwich (8 × 7.5 cm)	10 units	28-9909-83
Protran BA85, 0.45 µm blotting sandwich (8 × 7.5 cm)	10 units	28-9909-93
Membranes		
Hybond™ ECL (20 × 20 cm)	10 sheets	RPN2020D
Hybond ECL (8 × 7.5 cm)	10 sheets	RPN7.58D
Hybond-LFP (20 × 20 cm)	10 sheets	RPN2020LFP
Hybond-LFP (8 × 7.5 cm)	10 sheets	28-9909-84
Hybond-P (20 x 20 cm)	10 sheets	RPN2020F
Hybond-P (8 × 7.5 cm)	10 sheets	28-9909-83

Amersham Blocking agents

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

Amersham ECL HRP-linked Secondary Antibodies

Product	Quantity	Code no.
ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	1 ml	NA931-1ML
ECL Human IgG, HRP-Linked Whole Ab (from sheep)	1 ml	NA933-1ML
ECL Rabbit IgG, HRP-Linked Whole Ab (from donkey)	1 ml	NA934-1ML
ECL Mouse IgG, HRP-Linked $F(ab)_2$ fragment (from sheep)	1 ml	NA9310-1ML
ECL Rabbit IgG, HRP-Linked $F(ab)_2$ fragment (from donkey)	1 ml	NA9340-1ML
ECL Plex™ goat-α-mouse IgG-Cy2	150 µg	28-9011-08
ECL Plex goat-α-rabbit IgG-Cy2	150 µg	28-9011-10
ECL Plex goat-α-rabbit IgG-Cy3	150 µg	28-9011-06
ECL Plex goat-α-mouse IgG-Cy3	150 µg	PA43009
ECL Plex goat-α-rabbit IgG-Cy5	150 µg	PA45011
ECL Plex goat-α-mouse IgG-Cy5	150 µg	PA45010

Detection reagents

Product	Quantity	Code no.
Amersham ECL Select Western blotting reagents	for 1000 cm^2 membrane	RPN2235
Amersham ECL Prime Western blotting reagents	for 1000 cm^2 membrane	RPN2232

Product	Quantity	Code no.
Amersham ECL Western blotting reagents	for 1000 cm^2 membrane	RPN2109
Amersham ECL Western blotting reagents	for 4000 cm^2 membrane	RPN2106
Amersham ECL Western blotting reagents	for 6000 cm^2 membrane	RPN2134

Amersham ECL Plex CyDye conjugated Antibodies

Product	Quantity	Code no.
Amersham ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond ECL)	1	RPN998
Amersham ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond- LFP) for two slab gels	1	RPN999

Autoradiography Films

Product	Quantity	Code no.
Amersham Hyperfilm™ ECL (5 x 7 inches)	50 sheets	28-9068-35
Amersham Hyperfilm ECL (18 x 24 cm)	50 sheets	28-9068-36

Imaging Systems

Product	Quantity	Code no.
Typhoon Variable Mode Imagers		
Typhoon FLA 9500	1	29-0040-80
Typhoon FLA 7000	1	28-9558-09
ImageQuant Imagers		
ImageQuant™ LAS 4000	1	28-9558-10
ImageQuant LAS 4010	1	28-9558-11
ImageQuant LAS 4000 mini	1	28-9558-13
ImageQuant LAS 500	1	29-0050-63

Software and Accessories

Product	Code no.
CD and getting started:	
ImageQuant TL 7.0 and ImageQuant TL SecurITy 8.0 Software package (with Getting Started Guide)	28-9380-94
Licenses for ImageQuant TL only:	
ImageQuant TL, single user license	28-9236-62
Licenses for ImageQuant TL v7.01 and IQTL SecurITy v8.0:	
ImageQuant TL 7.01 and ImageQuant TL SecurITy 8.0, single user license	28-9332-73

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