GE Healthcare
Life Sciences

Amersham ECL Plus Western Blotting Detection Reagents

An improved non-radioactive method for the detection of immobilized specific antigens conjugated to Horseradish Peroxidase labelled antibodies.

Product Booklet

Codes: RPN2132
RPN2133

Page finder

1. Legal

http://www.gelifesciences.com/illustra

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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. (See safety data sheet for specific advice).

Note: that the protocol requires the use of Hydrochloric acid.

Warning: Hydrochloric acid causes burns and is an irritant. Please follow the manufacturer's safety data sheet relating to the safe handling and use of this material.

2.2 Storage

On receipt all components should be stored in a refrigerator at 2–8ºC.

The ECL Plus™ reagents are sensitive to prolonged exposure to light. Long term storage of the individual reagent should be in the light-tight containers in which they are provided.

2.3 Expiry

The components of these products are stable for at least 3 months when stored under the recommended conditions.

2.4 Packaging

The ECL Plus reagents are provided in light-tight containers.

3. Components and other materials required

3.1 Components

PRPN2132

Solution A: ECL Plus substrate solution containing tris buffer, 100 ml.

Solution B: Stock Acridan solution in Dioxane and Ethanol, 2.5 ml. See safety data sheet supplied.

Sufficient for 1000 cm^2 membrane

RPN2133

RPN 2132 x 3

Sufficient for 3000 cm^2 membrane

3.2 Solutions

The chemical reagents required for these solutions are available from GE Healthcare and are detailed in the USB™ Ultrapure catalogue.

- **Phosphate buffered saline (PBS) pH7.5**

  11.5 g Di-sodium Hydrogen Orthophosphate Anhydrous (80 mM)
  2.96 g Sodium Dihydrogen Orthophosphate (20 mM)
  5.84 g Sodium Chloride (100 mM)
  Dilute to 1000 ml with distilled water.
  Check pH

- **Tris buffered saline (TBS) pH7.6**

  8 g Sodium Chloride
  20 ml 1 M Tris HCl, pH 7.6
  Dilute to 1000 ml with distilled water.
  Check pH

- **Diluent and wash buffer PBS-Tween™ (PBS-T) and TBS-Tween (TBS-T)**

  Dilute the required amount of Tween 20 in the corresponding buffer. A 0.1% Tween 20 concentration is suitable for most blotting applications.

Storage of buffers

All buffers should be stable for at least 3 months if prepared in advance and stored at room temperature, although

storage in a refrigerator may be necessary to avoid microbial spoilage. Do not use Sodium Azide as a bacteriocide.

3.3 Reagents

- Immunodetection reagents (for example, primary and secondary antibodies)
- ECL Blocking Agent (RPN2125)

4. Description

The ECL Plus Western blotting detection reagents from GE Healthcare provide an improved non-radioactive method for the detection of immobilized specific antigens conjugated to Horseradish Peroxidase (HRP) labelled antibodies.

4.1 Chemiluminescent signal

Existing chemiluminescent detection reagents, such as ECL™ Western blotting are based on the oxidation of the cyclic Diacylhydrazide, luminol(1,2). ECL Plus utilizes a new technology, developed by Lumigen Inc, based on the enzymatic generation of an acridinium ester, which produces a more intense light emission of longer duration(3,4).

Combined HRP and peroxide catalyzed oxidation of the Lumigen PS-3 Acridan substrate generates thousands of acridinium ester intermediates per minute. These intermediates react with peroxide under slight alkaline conditions to produce a sustained, high intensity chemiluminescence with maximum emission at a wavelength of 430 nm(5) (see Figure 1). The resulting light is detected on autoradiography film (Hyperfilm™ ECL) or CCD camera.

ECL Plus Western blotting detection is optimized for use with Hybond™-P PVDF membrane where the performance over ECL Western blotting is most enhanced, but is also compatible with Hybond ECL nitrocellulose membrane.

The sensitivity increase(6) over ECL Western blotting may be between 4 and 20 fold, depending on the immunodetection system being used. In addition, the duration of signal from ECL Plus is extended, when used in combination with PVDF membrane, allowing successful exposures to be made up to 24 hours after initiation of the detection reaction. Exposures taken at this time point would need to be extended to 2–3 hours.

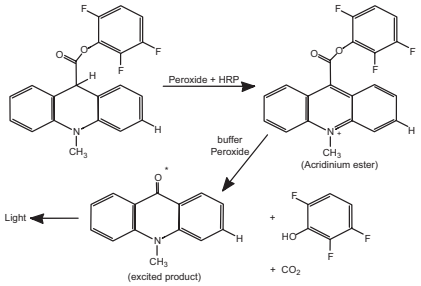

Fig 1. Chemiluminescent reaction of Lumigen PS-3 with horseradish peroxidase

4.2 Chemifluorescent signal

The chemistry of the light producing reaction with ECL Plus enables the reagents to be scanned on an instrument such as the Molecular Dynamics™ Storm™ 860. This is possible due to the generation of a fluorescent intermediate in the light producing reaction pathway with excitation of 430 nm and emission of 503 nm. ECL Plus provides excellent sensitivity with the versatility to allow use of the same Western blot for both film exposure and instrument scanning for quantification.

5. Critical parameters

- Read the entire protocol thoroughly before using the kit.
- ECL Plus can be used with both nitrocellulose and PVDF membranes both of which will give improvements in sensitivity over ECL. However the improvement observed is likely to be more significant with PVDF membranes than with nitrocellulose. In addition the prolonged light output is a feature of ECL Plus detection with PVDF membranes. Therefore in order to achieve the best results with ECL Plus reagents the use of PVDF membranes is recommended.
- ECL Plus is an extremely sensitive system. For results showing the best signal to noise ratio, it is essential to optimize the concentrations of both primary and secondary antibodies. Higher dilutions of antibodies are likely to be required when using ECL Plus in place of ECL Western blotting, particularly in association with PVDF membranes.
- During immuno-detection, sufficient solution should be used to adequately cover the membrane. Containers should be agitated gently on a mixer platform.
- While non-fat dried milk is strongly recommended as the membrane blocking agent, Gelatin, Casein and Bovine Serum Albumin (BSA) may also be used as alternative blocking reagents with the ECL Plus system.
- When washing, the volume of wash buffer should be as large as possible; 4 ml of buffer per cm^2 of membrane is suggested. Brief rinses of the membrane in wash buffer before incubating will improve washing efficiency.
- It is advisable to avoid the use of containers that are polystyrene based to mix ECL Plus reagents as the solution will turn milky and produce a precipitate. Other types of containers, such as

polypropylene, poly-ethylene, polymethyl-pentene and glass are all suitable for use.

- If exposure times of less than 5 seconds are routinely required, it is recommended that the antibodies used are further diluted as it is difficult to perform such short exposures.
- Although the working mix of the ECL Plus reagents is stable for 2 to 4 hours, it is recommended that reagents are mixed immediately before use. In the event that mixed reagents need to be left before use, protect from light by wrapping the container in foil or by storing in the dark.
- A film exposure showing similar sensitivity levels to that seen initially can be achieved 24 hours after substrate application. To do this the exposure time should be increased to 2 to 3 hours. However, if the original signal was very weak, detection may not be possible.

6. Quality control

Every batch of ECL Plus is functionally tested in a Western blotting application to ensure minimal batch to batch variability.

7. Protocol

7.1 Electrophoresis and blotting

Protocol	Notes
7.1.1. Perform electrophoresis and blotting according to usual techniques. Proteins should be transferred to Hybond-P PVDF or Hybond ECL for optimum results. Blots may be used immediately or stored in a desiccator at 2–8°C for up to 3 months.	**7.1.1.** Hybond-P PVDF should be pre-wetted in 100% methanol, washed in distilled water for 5 minutes and equilibrated in transfer buffer for at least 10 minutes before blotting. Hybond ECL should be pre-wetted in distilled water and equilibrated in transfer buffer for at least 10 minutes before blotting. ECL Plus is also suitable for use with supported nitrocellulose such as Hybond-C Extra. This membrane should be prepared as for Hybond ECL.

7.2 Blocking the membrane

Protocol	Notes
7.2.1. Block non-specific binding sites by immersing the membrane in 5% non-fat dried milk, 0.1% (v/v) Tween 20 in PBS or TBS (PBS-T or TBS-T, see page 5) for 1 hour at room temperature on an orbital shaker. Alternatively, membranes may be left in the blocking	**7.2.1.** The combination of non-fat dried milk and Tween should be sufficient for most applications. Optimum Tween concentrations will vary to suit specific experiments, but a 0.1% Tween 20 concentration is suitable for most blotting applications.

Protocol	Notes
7.2.1. *continued* solution overnight in a refrigerator at 2–8°C, if more convenient.	
7.2.2. Briefly rinse the membrane with two changes of wash buffer (see page 5).	**7.2.2.** While washing prepare the diluted primary antibody (step 7.3.1.).

7.3 Primary antibody incubation

Protocol	Notes
7.3.1. Dilute the primary antibody in PBS-T or TBS-T. The dilution factor should be determined empirically for each antibody.	**7.3.1.** Optimization of the antibody dilution can be performed by dot blot analysis. (see page 21).
7.3.2. Incubate the membrane in diluted primary antibody for 1 hour at room temperature on an orbital shaker.	**7.3.2.** Incubation times and temperatures may vary and should be optimized for each antibody. The conditions indicated are recommended starting points.
7.3.3. Briefly rinse the membrane with two changes of wash buffer and then wash the membrane in >4 ml/cm^2 of wash buffer for 15 minutes at room temperature.	
7.3.4. Wash the membrane for 3 x 5 minutes with fresh changes of wash buffer at room temperature.	**7.3.4.** While washing prepare the diluted secondary antibody (step 7.4.1.).

7.4. Secondary antibody incubation

Protocol	Notes
7.4.1. Dilute the HRP labelled secondary antibody or biotinylated antibody in PBS-T or TBS-T. The dilution factor should be determined empirically for each antibody (see page 21).	**7.4.1.** Use either an appropriate HRP labelled secondary antibody or a biotinylated secondary antibody and the HRP labelled streptavidin bridge system.
7.4.2. Incubate the membrane in the diluted secondary antibody for 1 hour at room temperature on an orbital shaker.	**7.4.2.** Incubation times and temperatures may vary and should be optimized for each antibody. The conditions indicated are recommended starting points.
7.4.3. Briefly rinse the membrane with two changes of wash buffer and then wash the membrane in > 4 ml/cm^2 of wash buffer for 15 minutes at room temperature.	
7.4.4. Rinse the membrane for 3 x 5 minutes with fresh changes of wash buffer at room temperature.	**7.4.4.** If using an HRP labelled secondary antibody proceed directly to step 7.6. (detection) after this wash procedure. If using a biotinylated antibody, while washing, prepare the diluted streptavidin HRP conjugate or complex (step 7.5.1.).

7.5. Streptavidin bridge incubation

Protocol	Notes
7.5.1. Dilute the Streptavidin HRP conjugate or Streptavidin-biotinylated HRP complex in PBS-T or TBS-T.	**7.5.1.** The dilution factor should be determined empirically (see page 21).
7.5.2. Incubate the membrane in the dilution for 45–60 minutes at room temperature on an orbital shaker.	
7.5.3. Briefly rinse the membrane with two changes of wash buffer and then wash the membrane in > 4 ml/cm^2 of wash buffer for 15 minutes at room temperature.	
7.5.4. Rinse the membrane for 3 x 5 minutes with fresh changes of wash buffer at room temperature.	

7.6. Detection

Protocol	Notes
7.6.1. Remove the detection reagents from storage at 2–8°C and allow to equilibrate to room temperature before opening.	

Protocol	Notes
7.6.2. Mix detection solutions A and B in a ratio of 40:1 (for example, 2 ml solution A + 50 µl solution B). The final volume of detection reagent required is 0.1 ml/cm^2.	**7.6.2.** If the mixed reagent is not to be used immediately protect it from exposure to the light either by wrapping in foil or storing in a dark place.
7.6.3. Drain the excess wash buffer from the washed membranes and **place protein side** up on a sheet of SaranWrap™ or other suitable clean surface. Pipette the mixed detection reagent on to the membrane.	**7.6.3.** The reagents should cover the entire surface of the membrane, held by surface tension on to the surface of the membrane.
7.6.4. Incubate for 5 minutes at room temperature.	
Chemiluminescent detection	
7.6.5. Drain off excess detection reagent by holding the membrane gently in forceps and touching the edge against a tissue. Place the blots protein side down on to a fresh piece of SaranWrap, wrap up the blots and gently smooth out any air bubbles.	**7.6.5.** Close the SaranWrap around the membrane to form an envelope or use an alternative, suitable detection pocket. Avoid applying pressure on to the membrane.
7.6.6. Place the wrapped blots, protein side up, in an x-ray film cassette.	**7.6.6.** Ensure there is no free detection reagent in the cassette; the film must not get wet.

Protocol	Notes
7.6.7. Place a sheet of autoradiography film (for example, Hyperfilm ECL) on top of the membrane. Close the cassette and expose for 15 seconds.	**7.6.7.** This stage should be carried out in a dark room using red safe lights. Do not move the film whileit is being exposed.
7.6.8. Remove the film and replace with a second sheet of unexposed film. Develop the first piece of film immediately, and on the basis of its appearance estimate how long to continue the exposure of the second piece of film. Second exposures can vary from 1 minute to 1 hour.	**7.6.8.** The detected blots can also be exposed to Polaroid™ film using the ECL mini-camera (RPN 2069), which is specifically designed for blots generated from mini-gel apparatus. The ECL mini-camera is suitable for blots up to 52 x 77 mm. Images can also be acquired using a CCD camera such as ImageMaster™ VDS-CL (18-1130-55).
Chemifluorescent detection	
7.6.9. Drain off excess detection reagent by holding the membrane gently in forceps and touching the edge against a tissue.	
7.6.10. On the Storm Imager, place the blot protein side down on the scanning bed. Cover with a fresh piece of SaranWrap and gently smooth out any air bubbles.	**7.6.10.** To help minimize air bubbles, a small amount of water should be placed on the scanning bed prior to applying the blot. The blot can be wrapped in SaranWrap for scanning, however any creases

Protocol	Notes
	7.6.10. *continued* in the SaranWrap will be visible on the scanned image. Other types of wrap or detection folder may cause loss of signal or may themselves fluoresce. Ensure that the blot does not dry out during or between scans. If the blot dries out, higher background noise will occur.
7.6.11. Scan using the blue fluorescence / chemifluorescence mode, 100 microns, PMT between 650 and 1000 v.	**7.6.11.** To ensure the best signal intensity, it is recommended that the blot should be scanned straight after substrate application. However, signal will still be visible on the following day but at a reduced level.

8. Additional information

8.1. Stripping and reprobing membranes - Chemiluminescent signal

The complete removal of primary and secondary antibodies from the membrane is possible following the protocol outlined below. The membranes may be stripped of bound antibodies and reprobed several times. Membranes should be stored wet wrapped in SaranWrap in a refrigerator (2–8°C) after each immunodetection.

Protocol	Notes
8.1.1. Submerge 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.	**8.1.1.** If more stringent conditions are required the incubation can be performed at 70°C or incubate for a longer time.
8.1.2. Wash the membrane for 2 x 10 minutes in PBS-T or TBS-T at room temperature using large volumes of wash buffer.	**8.1.2.** Membranes may be incubated with ECL Plus detection reagents and exposed to film to ensure removal of antibodies.
8.1.3. Block the membrane in 5% non-fat dried milk in PBS-T or TBS-T for 1 hour at room temperature.	
8.1.4. Repeat the immunodetection protocol, stages 7.3. to 7.6.	

8.2. Stripping and reprobing membranes - chemifluorescent signal

The complete removal of primary and secondary antibodies from membranes is possible, but removal of the fluorescent precipitate is only possible for PVDF membranes. The treatment required is too harsh for nitrocellulose and will either destroy or extensively damage the membrane.

Membranes may be stripped of fluorescent signal and bound antibodies, then reprobed several times but, as with all stripping procedures, loss of antigen may occur. Membranes should be stored wet wrapped in SaranWrap in a refrigerator (2–8°C) after each immunodetection.

Protocol	Notes
8.2.1. Gently agitate the membrane in 100% acetonitrile for 10 minutes.	**8.2.1.** If the initial signal was very strong, remove the blot from the acetonitrile and rinse briefly in wash buffer. Re-scan the blot to check if any signal is still present. If there is still signal, replace the blot in acetonitrile and agitate for a further 10 minutes.
8.2.2. Submerge 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.	**8.2.2.** If more stringent conditions are required the incubation can be performed at 70°C or incubated for a longer time. Membranes may be incubated with ECL Plus detection reagents and rescanned to ensure the removal of antibodies.

Protocol	Notes
8.2.3. Wash the membrane for 2 x 10 minutes using large volumes of wash buffer.	
8.2.4. Block the membrane in 5% non-fat dried milk in PBS-T or TBS-T for 1 hour at room temperature.	
8.2.5. Repeat the immunodetection protocol, stages 7.3. to 7.6.	

8.3. Determination of optimum antibody concentration

Due to the improved sensitivity of the ECL Plus detection reagents, optimization of antibody concentrations is recommended to ensure the best results. In general, lower concentrations of both primary and secondary antibodies are required with ECL Plus compared to ECL Western blotting, especially when using PVDF membranes.

Outlined below are protocols for determining optimal antibody concentrations.

8.3.1. Primary antibodies

Dot blots are 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.

8.3.1.1. Spot a suitable amount of protein sample on to a nitrocellulose or PVDF membrane and allow to air dry. Prepare one blot for each primary antibody dilution to be tested.

8.3.1.2. Incubate in blocking solution for 1 hour at room temperature with agitation.

8.3.1.3. Rinse the membranes briefly with two changes of wash buffer.

8.3.1.4. Prepare several dilutions of primary antibody: e.g.
nitrocellulose 1/1000, 1/2500, 1/5000, 1/10 000.
PVDF 1/5000, 1/10 000, 1/15 000, 1/20 000.
Incubate 1 blot in each dilution for 1 hour at room temperature with agitation.

8.3.1.5. Rinse blots in two changes of wash buffer, then wash for 1 x 15 minutes and 3 x 5 minutes in fresh changes of wash buffer.

8.3.1.6. Dilute the secondary antibody (using only one concentration) and incubate the membranes for 1 hour at room temperature with agitation.

8.3.1.7. Wash as detailed in step **8.3.1.5.**

8.3.1.8. Detect using ECL Plus detection reagents as detailed in step 7.6. of the protocol. The antibody dilution which gives the best signal with the minimum background should be selected.

8.3.2. Secondary antibodies

8.3.2.1. Prepare dot blots and block the membranes as detailed in **8.3.1.1.** and **8.3.1.2.**

8.3.2.2. Incubate in diluted primary antibody for 1 hour at room temperature with agitation.

8.3.2.3. Wash as detailed in step **8.3.1.5.**

8.3.2.4. Prepare several dilutions of secondary antibody: e.g.
nitrocellulose 1/10 000, 1/25 000, 1/50 000, 1/100 000,
PVDF 1/25 000, 1/50 000, 1/100 000, 1/200 000.
Incubate 1 blot in each dilution for 1 hour at room temperature with agitation.

8.3.2.5. Wash as detailed in step **8.3.1.5.**

8.3.2.6. Detect using ECL Plus detection reagents as detailed in step 7.6. of the protocol. The antibody dilution which gives the best signal with minimum background should be selected.

9. Troubleshooting guide

Problems	Possible causes / remedies
No signal	Check that transfer equipment is working properly and that the correct procedure has been followed. Check protein transfer by staining the gel and/or membrane. Some antigens may be affected by the treatments required for electrophoresis. Target protein degradation may occur if the blots are stored incorrectly. ECL Plus detection reagents may have become contaminated. Incorrect storage of the ECL Plus detection reagents may cause a loss of signal.
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. Film exposure time may have been too short.
Excessive, diffuse signal	Too much protein was loaded on to the gel. Electrophoresis and transfer protocols may need optimization.

Problems	Possible causes / remedies
Excessive, diffuse signal. continued	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.
Uneven, spotted background	Blotting technique requires optimization. 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.
High backgrounds	The concentrations of primary and secondary antibodies could be too high; optimization is required. Contamination can be transferred to the blots from electrophoresis and related equipment used in blot preparation. Transfer and incubation buffers may have become contaminated and require replacing. The blocking agent used was not freshly prepared or was too dilute or was incompatible with the application.

Problems	Possible causes / remedies
High backgrounds. continued	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.
	The type of membrane used was not compatible with non-radioactive systems.
	The post antibody washes were not performed for a sufficient period of time or were nor performed in a high enough volume.
	There was insufficient Tween in the post antibody washes.
	Insufficient changes of post antibody washes were used.
	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.

10. Related products

SDS-PAGE Electrophoresis Chemicals
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Low-range Rainbow™ MW Markers, Natural 45, 30, 20.1, 14.3, 6.5, 3.5 and 2.5 kDa	RPN755
High-range Rainbow MW Markers, Natural 220, 97, 66, 45, 30, 20.1 and 14.3 kDa	RPN756
Full-range Rainbow MW Markers, Recombinant 250, 160, 105, 75, 50, 35, 30, 25, 15 and 10 kDa	RPN800
ECL Western Blotting MW Markers, Biotinylated 97, 66, 45, 30, 20.1 and 14.3 kDa	RPN2107
Hybond ECL Membrane (nitrocellulose, pore size 0.45 mm) 20 x 20 cm, pack of 10 sheets	RPN2020D
Hybond ECL Membrane (nitrocellulose, pore size 0.2 mm) 30 cm x 3 m, 1 roll	RPN3032D
Hybond-P Membrane (PVDF, pore size 0.45 mm) 20 x 20 cm, pack of 10 sheets	RPN2020F
Hybond-P Membrane (PVDF, pore size 0.45 mm) 20 cm x 3 m, 1 roll	RPN203F
Hybond-C Extra Membrane (supported nitrocellulose, pore size 0.45 mm) 20 x 20 cm, pack of 10 sheets	RPN2020E
Hybond Blotting Paper 20 x 20 cm, pack of 100 sheets	RPN6101M
ECL Blocking Agent, 40 g	RPN2125

Mouse IgG, Horseradish Peroxidase Linked Whole Antibody (from sheep), 1 ml and 100 ml	NA931
Human IgG, Horseradish Peroxidase Linked Whole Antibody (from sheep), 1 ml	NA933
Rabbit IgG, Horseradish Peroxidase Linked Whole Antibody (from donkey), 1 ml and 100 ml	NA934
Rat IgG, Horseradish Peroxidase Linked Whole Antibody (from goat), 1 ml	NA935
Mouse IgG, Horseradish Peroxidase Linked Whole Antibody (from sheep) General Purpose Screening Reagent, 1 ml	NXA931
Mouse IgG, Horseradish Peroxidase Linked $F(ab')_2$ Fragment (from sheep), 1 ml	NA9310
Human IgG, Horseradish Peroxidase Linked $F(ab')_2$ Fragment (from sheep), 1 ml	NA9330
Rabbit IgG, Horseradish Peroxidase Linked $F(ab')_2$ Fragment (from donkey), 1 ml	NA9340
Rat IgG, Horseradish Peroxidase Linked $F(ab')_2$ Fragment (from goat), 1 ml	NA9350
Mouse IgG, Biotinylated Whole Antibody (from sheep), 2 ml	RPN1001
Human IgG, Biotinylated Whole Antibody (from sheep), 2 ml	RPN1003
Rabbit IgG, Biotinylated Whole Antibody (from donkey), 2 ml	RPN1004
Rat IgG, Biotinylated Whole Antibody (from goat), 2 ml	RPN1005
Immunoprecipitation Starter Pack	17-6002-35

Streptavidin-biotinylated Horseradish Peroxidase Complex	RPN1051
Streptavidin Horseradish Peroxidase Conjugate	RPN1231
ECL Western Blotting System	RPN2108
ECL Western Blotting Detection Reagents	
For 1000 cm^2 membrane	RPN2109
For 2000 cm^2 membrane	RPN2209
For 4000 cm^2 membrane	RPN2106
For 6000 cm^2 membrane	RPN2134
ECL Glycoprotein Detection Module 25 Membrane Reactions Order ECL Detection Reagents separately	RPN2190
ECL Protein Biotinylation Module Order ECL Detection Reagents separately	RPN2202
ECL Protein Biotinylation System For 2000 cm^2 membrane	RPN2203
ECL Phosphorylation Module Sufficient for 25 blots Order ECL Detection Reagents separately	RPN2220
Hypercassette™	
18 x 24 cm	RPN11642
30 x 40 cm	RPN11644
10 x 12 inches	RPN11650
5 x 7 inches	RPN11648
Hypertorch™, Red Light Darkroom Torch	RPN1620
Sensitize™ Pre-flash Unit	RPN2051
Hyperfilm ECL	
18 x 24 cm, pack of 25 films,	RPN2103
30 x 40 cm, pack of 25 films,	RPN2104

10 x 12 inches, pack of 25 films,	RPN1681
5 x 7 inches, pack of 25 films,	RPN1674
Hyperprocessor™ Automatic Film Processor (not available in all countries)	
220/240 V	RPN1700
110/120 V	RPN1700A
ECL Mini-camera	RPN2069
ImageMaster VDS-CL, CCD Camera	18-1130-55
Storm 860 and ImageQuant	860-PC

11. References

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RPN2132PL AE 02-2011