GE Healthcare
Life Sciences

Amersham Hybond-P PVDF Membrane

optimized for protein transfer

Product Booklet

Codes: RPN2020F
RPN1416F
RPN303F
28990983

Page finder

1. Legal

First published 2001.

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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.

We recommend that this product and components are 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. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes wash immediately with water.

Note that the procedures require the use of: Acrylamide/ NN,–methylene–bis-acrylamide: toxic substance Ammonium persulfate: harmful TEMED: highly flammable, irritant -Mercaptoethanol: poisonous substance

Methanol: toxic substance: highly flammable Sodium dodecyl sulfate: irritant Caution:This product may be used with radioactive materials. Please follow the manufacturer's Safety Data Sheet relating to the safe handling and use of these materials.

2.2. Storage

Membranes should be stored in a clean, dry atmosphere away from noxious fumes. In order to preserve optimum performance, avoid conditions of extreme humidity.

2.3. Stability

Before opening, membranes are stable for one year. Once open keep in the bags in which they were received. Performance is consistent for up to twelve months when stored under the recommended conditions.

3. Components

RPN2020F
20 × 20 cm, 10 sheets

RPN1416F
14 × 16 cm, 15 sheets

RPN303F
30 cm × 3 m, 1 roll

28990983
8 × 7.5 cm, 10 sheets

4. Other materials required

Equipment

- Polyacrylamide electrophoresis apparatus, GE Healthcare Mini VE system, 80–6418–58
- Electroblotting cassette, for example GE Healthcare Blot module, 80–6418–96
- Hybond Blotting Paper
- Trays/dishes
- Assorted laboratory glassware
- Orbital shaker
- SaranWrap™ or similar cling film
- Timer
- Detection facilities
- X–ray film cassette, for example, Hypercassette™
- Autoradiography film, for example, Hyperfilm™ ECL™, Hyperfilm MP
- X–ray film developing facilities.
- Suitable scanner platforms, for example Storm™ or FluorImager™ from Molecular Dynamics™ Inc.

Solutions

Reagents required for electrophoresis, blotting and immunodetection of proteins

All reagents should be of AnalaR™ grade where possible.

Protein sample loading buffer	4.0 ml Distilled water 1.0 ml 0.5 M Tris-HCl, pH 6.8 0.8 ml Glycerol 1.6 ml 10% (w/v) Sodium dodecyl sulfate (SDS)

Protein sample loading buffer	0.4 ml –Mercaptoethanol 0.5 ml 0.05% (w/v) Pyronin Y (e.g. Sigma™ code P-7017) Store in dark at room temperature for a maximum of 2 weeks.
0.5M Tris-HCl, pH 6.8	6.0 g Tris base Make up to a final volume of 100 ml after adjusting pH to 6.8 with hydrochloric acid. Store at 2–8°C.
Resolving gel buffer	90.8 g Trizma™-base 2.0 g Sodium dodecyl sulfate Add approximately 900 ml distilled water. Mix to dissolve. Adjust pH to 8.8 with hydrochloric acid. Make up to a final volume of 100 ml. Store at 2–8°C.
Acrylamide stock solution, 30% (w/v)	30.0 g Acrylamide 0.8 g N,N -methylene-bis-acrylamide Add to approximately 50 ml of distilled water. Mix to dissolve. Make up to a final volume of 100 ml. Store at 2–8°C for a maximum of 2 weeks. **Warning:** Acrylamide and N,N -methylene-bis-acrylamide are toxic. Please follow the manufacturer's instructions relating to the safe handling and use of these materials.
Ammonium persulfate stock solution, 10% (w/v)	0.1 g Ammonium persulfate 1.0 ml Distilled water Mix well. Prepare fresh as required.

Resolving gel	7.5 ml Acrylamide stock solution, 30% (w/v) 9.0 ml Resolving gel buffer 2.0 ml Distilled water 180 µl Ammonium persulfate stock solution. 10% (w/v) 18 µl N,N,N ,N -Tetramethylethylenediamine (TEMED)
Stacking gel (4%)	1.8 ml Acrylamide stock solution, 30%(v/v) 5.0 ml Stacking gel buffer 4.0 ml Distilled water 50 µl Ammonium persulfate stock solution, 10% (w/v) 10 µl N,N,N,N-Tetramethylethylenediamine (TEMED)
Stacking gel buffer	30.3 g Trizma base 2.0 g Sodium dodecyl sulfate Add approximately 900 ml of distilled water. Mix to dissolve. Adjust pH to 6.8 with hydrochloric acid. Make up final volume to 1000 ml. Store at 2–8°C.

Protein transfer buffer	3.03 g Trizma-base 14.4 g glycine 200 ml methanol (optional) Add approximately 650 ml of distilled water. Mix to dissolve. Make up to a final volume of 1000 ml. Store at 2–8°C. Inclusion of methanol in the buffer minimizes swelling of the gel during blotting.
Tris buffered saline (TBS), pH 7.6	12.1 g Trizma-base 40.0 g Sodium chloride Dilute to 5000 ml with distilled water. Adjust pH to 7.6 with hydrochloric acid. Store at 2–8°C.
TBS-Tween (TBS-T)	Dilute required volume of Tween™ 20 in TBS to give a 0.1% (v/v) solution. Store at 2–8°C.
Phosphate buffered saline (PBS), pH 7.5	11.5 g Di-sodium hydrogen orthophosphate, anhydrous 2.96 g Sodium dihydrogen orthophosphate 5.84 g Sodium chloride Dilute to 1000 ml with distilled water. Adjust pH to 7.5. Store at 2–8°C.
PBS-Tween (PBS-T)	Dilute required volume of Tween 20 in PBS to give a 0.1% (v/v) solution. Store at 2–8°C.

5. Description

All Hybond™ membranes from GE Healthcare are manufactured specifically for Life Science applications. Production runs are carefully controlled and the product exhaustively screened to ensure that only the most consistent product reaches the user.

All GE Healthcare membranes are identical on both sides.

Hybond–P is a hydrophobic polyvinylidene difluoride membrane optimized for protein transfer. It offers the following features:

- high mechanical strength
- high protein binding capacity
- may be used for protein staining and immunoblotting
- chemical stability allowing use of a range of solvents for rapid destaining.

It is particularly recommended for use with:

- ECL Glycoprotein detection system from GE Healthcare
- ECL reagents
- ECL Plus™ reagents

and gives excellent results with: ECF™ substrates and detection system and colorimetric detection reagents such as:

- Colloidal gold
- BCIP/NBT substrate
- DAB substrate

6. Critical parameters

Storage

Hybond-P should be stored in a dry and clean environment. Membranes are affected by the environment and so should be kept in the bags and boxes in which they are received.

Handling

Membranes should be handled wearing gloves or using blunt ended forceps. All membranes should be cut using clean sharp scissors to avoid damage to the membrane edges.

Pre-wetting

Hybond-P should be pre-wet with methanol before use followed by washing in distilled water and equilibration in an appropriate buffer. See page 12 for full wetting instructions.
If Hybond-P dries out at any time the wetting procedure must be repeated.

7. Blotting protocols

7.1. Protocol for Western blotting

There are three basic methods for transferring proteins to membranes, capillary blotting, diffusion blotting, and electroblotting. Capillary and diffusion blotting are relatively slow procedures. As electroblotting is by far the most widely used, this booklet limits itself to this procedure.

Protocol	Notes
1. Separate the protein samples using gel electrophoresis or isoelectric focusing.	
2. Remove the stacking gel and orientate the resolving gel by removing a corner.	
3. Soak the gel in the protein transfer buffer for at least 10–20 minutes.	**3.1.** For transfer buffers without methanol it is essential that complete equilibration of the resolving gel is achieved to prevent distortions within the gel which would cause band smearing. Only a brief rinse is required to achieve equilibration if the transfer buffer contains methanol.
4. Prepare a sheet of Hybond-P. Cut the membrane to size, a) pre-wet the membrane in 100% methanol (10 seconds),	**4.1.** Hybond-P must be kept wet at all times. Should it dry out, re-wet in methanol and water as described in

Protocol	Notes
4. *Continued* b) wash in distilled water 1 × 5 minutes, and then c) equilibrate in the protein transfer buffer for at least 10 minutes.	**4.1.** *Continued* step 4 of this protocol.
5. Assemble the electroblotting cassette and place between the electrodes in the blotting unit, according to the manufacturer's instructions, using the appropriate transfer buffer.	
6. Transfer for 1 hour at 100 V with cooling. This setting is suitable for gels 80 to 320 cm^2 in size.	**6.1.** It is recommended that cold transfer buffer is used. **6.2.** Extended transfer times of 2 hours at 80 V or 30 V overnight, are also suitable. Cooling is essential for best results.
7. Following transfer remove the membrane from the blotting cassette, mark the orientation of the gel on the membrane and rinse briefly in PBS. Trim the membrane/ blot.	**7.1.** Trimming the blot will prevent high background edge effects.

Protocol	Notes
8. Blots may be used immediately or stored.	**8.1.** Membranes should be air dried before storage. Blots may be stored between sheets of Hybond Blotting Paper wrapped in SaranWrap at 2–8°C for up to 3 months.Once dry the mem-brane will require pre-wetting before use see step 4, page 12.

7.2. Protocol for Immunodetection

There are a number of procedures available for the immunodetection of proteins, suitable reagents are available from GE Healthcare. A flow diagram (figure 1) demonstrates the possible uses of some of these.

During immunodetection sufficient solution should be used to adequately cover the membrane and the containers should be gently agitated. Details of a recommended rolling cylinder system can be found in Additional Information, page 26.

The protocol below is representative of the most commonly used procedures.

Protocol	Notes
1. Pre-wet membrane in 100% (v/v) methanol. Wash 1 × 5 minutes in distilled water.	**1.1.** If blots have been allowed to dry out or stored, the wetting procedure must be repeated.
2. Block non-specific binding sites on the membrane, using 0.1% (v/v) Tween 20, 5% (w/v) dried skimmed milk in PBS, for example. Incubate 1 hour. Briefly rinse the membrane twice with PBS-T.	**2.1.** Solutions that may be used for blocking include gelatin (0.25–3% (w/v)), BSA (0.25–3% (w/v)), normal serum, reconstituted dried skimmed milk (most commonly used) or Tween 20, in combination or alone. See Additional Information section. **2.2.** The choice of a PBS or TBS will depend on the detection system of choice. The use of PBS is not advised with

Protocol	Notes
	2.2. *Continued.* alkaline phosphatase based detection systems as phosphate ions are powerful inhibitors of this enzyme.
	2.3. As a general rule as large a volume as possible of wash buffer should be used, 1–2 ml/cm^2 is suggested.
3. Wash the blot with an excess volume of PBS-T. Wash 1 × 5 minutes.	**3.1.** The antigenicity of some antibodies may be affected by the presence of Tween 20. Washes can be altered, for example, if high background appears, increase number of washes.
4. Incubate with the primary antibody at the optimized dilution in PBS-T for 1 hour.	**4.1.** Blocking or carrier proteins may also be included, for example BSA (0.1–0.25% (w/v) or gelatin (0.25% (w/v)).
	4.2. The dilution of the primary antibody required to give optimal results will vary and must be determined for each antibody. See Additional Information section.

Protocol

5. Briefly rinse in PBS-T. Wash with excess PBS-T, 2 × 10 minutes.

6. Incubate with the second antibody at the appropriate dilution in PBS-T for 1 hour.

7. Briefly rinse in PBS-T. Wash with excess PBS-T, 3 × 10 minutes.

8. Proceed with one of the following detection systems, using an appropriate optimized dilution (see figure 1):

a ^{125}I labelled secondary antibodies
Dry the membrane and autoradiograph.

b Enzyme labelled secondary antibodies
Add appropriate enzyme substrate solution to the blot following the manufacturer's instructions.

c Biotinylated secondary antibodies
Proceed with one of the streptavidin based detection systems

Notes

8.1. ^{125}I labelled antibodies. Expose the dried sample to intensifying screens such as Hyperscreen™ and film such as Hyperfilm-MP at -70°C. Pre-flashing the film will increase its sensitivity to the signal and linearize its response. Full details of the procedure may be obtained with Sensitize™ flash gun.

8.2. Colorimetric detection. Substrate solutions which yield an insoluble reaction product include Diaminobenzidine (DAB) and 4-Chloro-1-napthol (QCIN) for use with peroxidase, and Bromo

Protocol	Notes
8. *Continued.* **i** ^{125}I streptavidin and autoradiograph **ii** Streptavidin gold. Incubate the blot in the reagent at a suitable dilution. **iii** Enzyme-labelled streptavidin conjugate, incubate with colour chemiluminescence or chemifluorescent substrates.	**8.2.** *Continued.* chloro indolyl phosphate/ Nitro blue tetrazolium (BCIP/ NBT) for use with alkaline phosphatase. Blots should be incubated at room temperature until the desired band intensity is achieved. In some cases sensitivity can be increased by rendering the membrane transparent, following the enzyme reaction and drying, by immersing in toluene or xylene. Reaction products must be insoluble in such organic solvents. **8.3.** Chemifluorescent detection In the presence of the appropriate enzyme (alkaline phosphatase) a chemifluorescent substrate, such as ECF, yields a fluorescent product which may be detected using fluorescence scanning instrumentation for example the Molecular Dynamics FluorImager and Storm.

Protocol	Notes
	8.4. Chemiluminescence detection In the presence of ECL and ECL Plus enzyme chemiluminescence, substrates produce light which can be captured on X-ray film, for example Hyperfilm ECL to give a hard copy result.

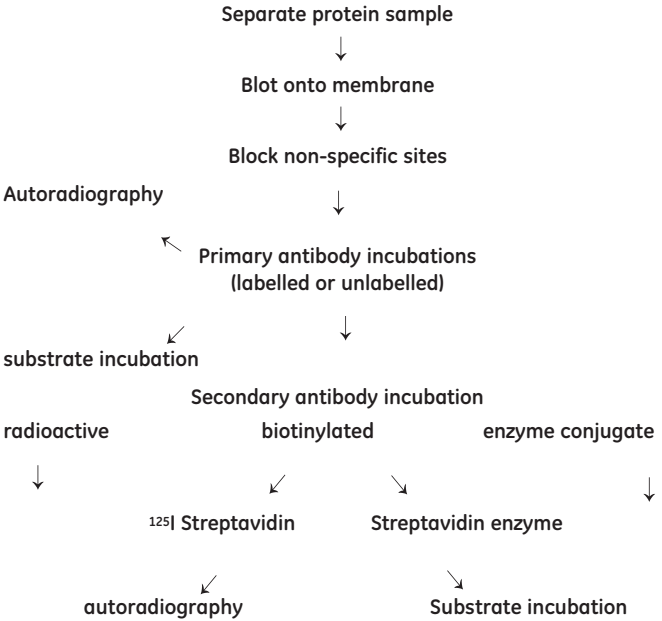

Figure 1. Flow diagram for protein blotting immunodetection

7.3. Protocol for dot blotting (manual)

The following is a general protocol for dot blotting proteins. A number of devices are also commercially available, for example the GE Healthcare slot blotter. These give a more consistent and even application of the sample than the manual procedure described below. This parameter is particularly important in those experiments requiring quantification.

Protocol	Notes
1. Cut the Hybond-P membrane to size.	
2. Using a pencil, mark the membrane lightly with a grid or dots to guide subsequent sample application. There should be a minimum distance of 1 cm between samples applied in a volume of 5 µl or less.	
3. Pre-wet the membrane in methanol, wash 1 × 5 minutes in distilled water, followed by TBS or PBS. Place the membrane on a sheet of PBS/TBS soaked Hybond Blotting Paper.	
4. Dilute the sample in an appropriate buffer, for example TBS or PBS to the required concentration. A sample size of 1–2 µl is ideal for manual dot blotting.	**4.1.** Carrier substances, for example bovine serum albumin, may be included in the diluent buffer to improve retention of very

Protocol	Notes
	4.1. *Continued.* small amounts of target on the membrane.
5. Carefully apply the 1–2 µl aliquot sample to the membrane, avoiding touching the membrane with the pipette tip. Place the membrane on a sheet of clean dry Hybond Blotting Paper and leave to air dry.	**5.1.** Apply samples directly to the damp membrane. Pools of PBS/TBS must be allowed to absorb before dotting. Keep membrane damp at all times. **5.2.** If the sample volume is greater than 2 µl, then apply in successive 2 µl aliquots to the same position on the membrane, allow the aliquot to absorb between each application. This will reduce sample spreading.
6. No further treatment is required for protein samples.	
7. Blots may be used immediately or stored at 2–8°C wrapped in SaranWrap for up to 3 months.	**7.1.** Pre wetting is required before further processing of the membrane can be undertaken.

7.4. Protocols for reprobing membranes

Following ECL, ECL Prime or ECL Plus detection it is possible to reprobe the membrane several times to either clarify or confirm results or when small or valuable samples are being analyzed. Sequential reprobing of membranes with a variety of antibodies is possible. The blots must be stored wet wrapped in SaranWrap at 2–8°C after each immunodetection.

Protocol one

This procedure is suitable for sequential reprobings using primary and secondary antibodies raised in different species using ECL systems or where different immunodetection systems have been used.

Protocol	Notes
1. Reapply prepared ECL detection reagents to the blots and re-expose to a sheet of autoradiography film.	**1.1.** Refer to detection protocol supplied with the reagents.
2. If a signal is detected incubate the blot in prepared ECL detection reagents for 30 minutes. Repeat step 1. If no signal is detected proceed to step 3.	**2.1.** Do not allow the blots to dry out. Excessive incubation in ECL reagents will inhibit peroxidase activity preventing the emission of light.
3. Perform immunodetection using a primary antibody raised in a different species to the first. Secondary antibodies must demonstrate no cross reactivity.	

Protocol two – stripping and reprobing membranes

The complete removal of primary and secondary antibodies from membranes is possible following the method outlined below. The membrane may be stripped of bound antibodies and reprobed several times. This procedure may not be suitable for enzyme substrates where insoluble reaction products are deposited on the membrane. Membranes should be stored wet wrapped in SaranWrap at 2–8°C after each immunodetection.

In excess of 50% of some target proteins can be lost when performing experiments where blots are stripped and reprobed. It is therefore important to consider which antigen is present in least abundance and probe for this first.

Protocol	Notes
1. Submerge the membrane in stripping buffer (100 mM -mercaptoethanol, 2% (w/v) sodium dodecyl sulfate, 62.5 mM Tris-HCl pH 6.7) and incubate at 60°C for 30 minutes with occasional agitation.	**1.1.** Different antibodies may require different stripping temperatures and/or incubation periods.
2. Wash the membrane for 2 × 10 minutes in TBS-T or PBS-T at room temperature using large volumes of wash buffer.	**2.1.** Membranes may be incubated with the ECL detection reagents and exposed to film to ensure removal of antibodies.
3. Block the membrane by immersing in 5% (w/v) blocking reagent in TBS-T or PBS-T for 1 hour at room temperature.	**3.1.** Refer to note 2.1. on page 15.
4. Perform immunodetection as described on page 15.	

8. Additional information

8.1. Determination of optimum antibody concentration

Due to the sensitivity of ECL Western blotting, optimization of antibody concentrations is necessary to ensure the best results. Generally, lower concentrations of both primary and secondary antibodies are required with ECL and ECF compared to colorimetric detection. Outlined below are protocols for determining optimal antibody concentrations.

Dot blots are a quick and effective method for 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.

1. Primary antibodies

1.1. Spot serial dilutions of the antigen on to prepared Hybond-P, and allow to dry. The dilution range should be representative of the likely quantity of antigen requiring detection. Prepare one blot for each primary antibody dilution to be tested. See Protocols section page 15.

1.2. Incubate in blocking solution (see page 15) for 1 hour at room temperature with agitation.

1.3. Wash the membranes briefly in two changes of washing buffer then wash once for 15 minutes and twice for 5 minutes, with fresh changes of the washing buffer at room temperature.

1.4. Prepare several dilutions of primary antibody, for example, 1/100, 1/500, 1/1000, 1/1500. Incubate one blot in each antibody dilution for 1 hour at room temperature.

1.5. Wash as detailed in step 1.3.

1.6. Dilute the secondary antibody and incubate the membranes for 1 hour at room temperature.

1.7. Wash as detailed in step 1.3.

1.8. Detect using the appropriate detection reagents as detailed on page 17.

1.9. The antibody dilution that gives the maximum signal with minimum background should be selected.

2. Secondary antibodies

2.1. Prepare dot blots and block the membranes as described in 1.1 and 1.2 above. Wash as detailed in step 1.3.

2.2. Incubate in an optimized dilution of primary antibody for 1 hour at room temperature.

2.3. Wash as detailed in step 1.3.

2.4. Prepare several dilutions of secondary antibody: for example 1/1500, 1/3000, 1/5000, 1/10 000, 1/50 000. Incubate one blot in each antibody dilution for 1 hour at room temperature.

2.5. Wash as detailed in step 1.3.

2.6. Detect using the appropriate detection reagents as detailed on page 21. The antibody dilution that gives maximum signal with minimum background should be selected.

8.2. Blocking buffers used in Western blotting

After transfer of proteins to membrane (for example Hybond-P), binding sites must be saturated to prevent non-specific binding of antibodies or other probes. Failure to efficiently block the membrane can lead to high background. Blocking is achieved by incubating the membrane in a relatively concentrated solution of heterologous protein or detergent.

The diversity of primary antibodies used for immunological screening, each displaying varying affinities for the target protein of interest, does not allow a standard protocol to be generally recommended. Many of the blocking agents used are a personal preference and simply changing blocking reagents may improve detection in specific circumstances. GE Healthcare recommends that a number of blocking solutions be tested for optimization on either Western blots or dot blots for the minimum background without loss of signal.

The following list of alternative blocking solutions may be used as a guide for the optimization of detection.

Blocking agent	Features
Dried milk For example, 5% (w/v) non-fat dried milk in PBS or TBS	Inexpensive Clean background Deteriorates rapidly Disguises some antigens
Milk/Tween 20 For example, 5% (w/v) non-fat dried milk, 0.1% (v/v) Tween-20 in PBS or TBS	Inexpensive Clean background Deteriorates rapidly Disguises some antigens
Tween 20 For example, 0.1% (v/v) Tween-20, 0.02% (w/v) sodium azide in PBS or TBS	Inexpensive Allows staining after antigen detection May give rise to residual background
BSA For example, 3% (w/v) BSA, 0.02% (w/v) sodium azide in PBS	Good signal strength Relatively expensive

Blocking agent	Features
Fish Gelatin For example 2% (w/v) Fish Gelatin	Relatively expensive Can be used at 4°C without gelling Easy to dissolve Can mask some proteins Contains some competitive reactants such as biotin
Horse serum For example, 10% (v/v) horse serum, 0.02% (w/v) sodium azide in PBS or TBS	Moderately expensive Clean background Incompatible with some anti-immunoglobulin antibodies

8.3. Details of the container recommended for immunodetection

For optimal processing of blots GE Healthcare recommends the use of a rolling cylinder. This technique can be used with any suitable container of sufficient length and circumference to accommodate the blot. The lid should seal well and fit such that while being rolled on its side on a roller incubator, the inner walls of the container are uniformly covered with solution. Blots are placed against the inner wall of the container with the side of the membrane carrying the transferred proteins facing into the centre. The natural spring of the membrane and, once wet, surface tension is sufficient to adhere the blot to the wall of the container.

The rolling cylinder procedure allows the use of the small volumes of reagents recommended in the protocol. Since processing is dynamic, i.e. the membrane is constantly moved through the reagent solution, no localized depletion of reagent solutions occurs and the washing steps are very thorough. This directly results in increased sensitivity

of detection and strength of signal, and reduced backgrounds. As the blot is effectively immobilized on the inner surface of the cylinder there is no risk of uneven detection, or scratching or tearing of the membrane during immunodetection.

A schematic representation of the processing technique is shown in Figure 2.

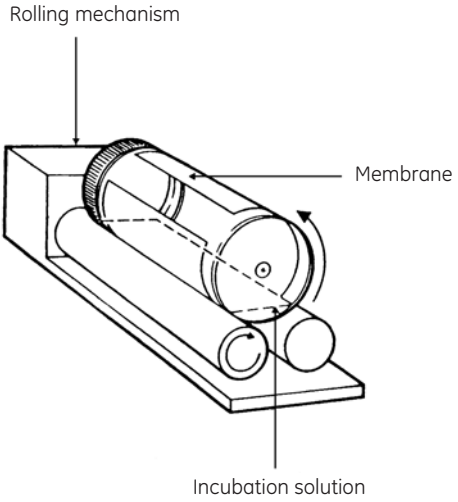

Figure 2. Immunodetection container

8.4. Recommended applications for blotting membranes

Hybond membranes for binding nucleic acid

Applications	Hybond-NX (nylon)	Hybond-XL (positively charged nylon)	Hybond-N+ (positively charged nylon)	Hybond-N (neutral nylon)
Southern blotting				
DNA fingerprinting	+	+	+	+
Radioactive	++	+++	++	++
ECL	-	-	+++	-
AlkPhos Direct™	-	-	+++	-
Gene Images™	-	-	+++	-
Alkali blotting/fixation	--	+++	++	--
Low volume hybridizations	+++	+++	+	--
Rapid-hyb™ buffer	+	+++	++	+
Northern blotting				
Radioactive	++	+++	+	++
Non-radioactive	-	-	++	-
Dot/slot blots				
Radioactive	++	+++	++	++
Non-radioactive	-	-	++	-
Colony/plaque lifts				
Radioactive	+++	++	+	++
Non-radioactive	+	-	++	+

Hybond membranes for binding protein

Applications	Hybond-P	Hybond-ECL	Hybond-C Extra
Western blotting			
ECLdetection	+++	+++	+
ECL Prime detection	+++	++	+
ECL Plus detection	+++	++	+
Chromogenic detection	++	++	+
Colloidal gold detection	++	++	-
ECF detection	+++	+	-
Radioactive detection	+	+	++
Glycoprotein detection	+++	+	+
Reprobing Westerns	+++	-	+
Expression screening	+	-	+++

Key: Suitable = +, Recommended = ++, Highly recommended = +++
Not recommended = -, Unsuitable = --

8.5. Related products

Hybond membranes for protein transfer

Size	Pack size	Code		
		Hybond-C Extra	Hybond ECL (pure nitro-cellulose)	Hybond-P (hydro-phobic PVDF)
82 mm diam	50 discs	RPN82E	RPN82D	
87 mm diam	50 discs			
132 mm diam	50 discs		RPN132D	
137 mm diam	50 discs	RPN137E	RPN137D	
6 × 8 cm	50 sheets		RPN68D	
7 × 8 cm	50 sheets		RPN78D	
8 × 7.5 cm	10 sheets		RPN7.58D	28990983
9 × 10.5 cm	10 sheets		RPN910D	
10 × 10 cm	10 sheets		RPN1010D	
14 × 16 cm	15 sheets			RPN1416F
15 × 20 cm	10 sheets		RPN1520D	
20 × 20 cm	10 sheets	RPN2020E	RPN2020D	RPN2020F
22 × 22 cm	10 sheets			
30 × 50 cm	5 sheets			
20 cm × 3 m	1 roll	RPN203E	RPN203D	
30 cm × 3 m	1 roll		RPN3032D	
30 cm × 3 m	1 roll	RPN303E	RPN303D	RPN303F

Hybond blotting paper

Size	Pack size	Code
46 × 57 cm	100 sheets	RPN6100M
20 × 20 cm	100 sheets	RPN6101M
23 × 100 cm	1 roll	RPN6102M
19 cm × 100 m	1 roll	RPN6103M
27 cm × 100 m	1 roll	RPN6104M

Hybond membranes for nucleic acid transfer

Size	Pack size	Code		
		Hybond-N (nylon)	Hybond-N+ (positively charged nylon)	Hybond-XL (positively charged nylon)
82 mm diam	50 discs	RPN82N	RPN82B	RPN82S
87 mm diam	50 discs	RPN87N	RPN87B	RPN87S
132 mm diam	50 discs	RPN132N	RPN132B	RPN132S
137 mm diam	50 discs	RPN137N	RPN137B	RPN137S
11.9 × 7.8 cm	50 sheets	RPN119N	RPN119B	RPN119S
22.2 × 22.2 cm	50 sheets		RPN2250B	RPN2222S
22.5 × 22.5 cm	50 sheets		RPN225B	
15 × 73 mm	50 sheets		RPN1567B	
12 × 10 cm	20 sheets	RPN1210N	RPN1210B	RPN1210S
15 × 10 cm	20 sheets	RPN1510N	RPN1510B	RPN1510S
15 × 20 cm	10 sheets	RPN1520N	RPN1520B	RPN1520S
20 × 20 cm	10 sheets	RPN2020N	RPN2020B	RPN2020S
22 × 22 cm	10 sheets	RPN2222N	RPN2222B	
30 × 50 cm	5 sheets	RPN3050N	RPN3050B	RPN3050S
20 cm × 3 m	1 roll	RPN203N	RPN203B	RPN203S
30 cm × 3 m	1 roll	RPN303N	RPN303B	RPN303S
82 mm	50 gridded discs		RPN1782B	
87 mm	50 gridded discs		RPN1787B	
132 mm	50 gridded discs		RPN1732B	
137 mm	50 gridded discs		RPN1737B	

Protein studies

ECL Western blotting detection reagents

for 4000 cm^2 membrane	RPN2106
for 2000 cm^2 membrane	RPN2209
for 1000 cm^2 membrane	RPN2109

ECL prime

for 1000 cm^2 membrane	RPN2232

ECL Plus

for 1000 cm^2 membrane	RPN2132
ECL Western blotting analysis system	RPN2108

For the detection of either mouse or rabbit membrane bound primary antibodies.

Sufficient for 1000 cm^2 membrane.

ECF Western blotting system

Applicable for use with fluorescence scanning systems

For the detection of either mouse or rabbit membrane bound primary antibodies.

Sufficient for 2500 cm^2 or 5000 cm^2 membrane.	RPN5781–RPN5784
Streptavidin-horseradish peroxidase conjugate	RPN1231

Horseradish peroxidase-labelled second antibody conjugates

Mouse Ig HRP-linked whole antibody (from sheep)	NA931
Rabbit Ig HRP-linked whole antibody (from donkey)	NA934
Human Ig HRP-linked whole antibody (from sheet)	NA933

ECL glycoprotein detection module	RPN2190/ 2191
ECL protein biotinylation module	RPN2202/ 2203
Molecular weight markers	
ECL protein molecular weight markers (molecular weight range 14 400 – 200 000 Da)	RPN2107
Rainbow™ coloured protein molecular weight markers (molecular weight range 2350 – 46 000 Da)	RPN755
Rainbow coloured protein molecular weight markers (molecular weight range 14 300 – 200 000 Da)	RPN756
Full range of Rainbow recombinant molecular weight markers (molecular weight range 10 000 – 250 000 Da)	RPN800

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