PerkinElmer™ Life Sciences, Inc.


# POLYSCREEN®
# PVDF TRANSFER MEMBRANE
# IMPROVED
# NEF1000
# NEF1002

**For Laboratory Use**
**CAUTION: A Research Chemical for Research Purposes Only**

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## IMPROVED POLYSCREEN® TRANSFER MEMBRANE INTRODUCTION

The PerkinElmer Life Sciences, Inc. PolyScreen® Transfer Membrane is a polyvinylidene fluoride (PVDF) microporous membrane for binding proteins that are transferred from a variety of gel matrices. This membrane is hydrophobic and offers a uniformly controlled pore structure with a high binding capacity for biomolecules. When compared to a nitrocellulose membrane, it has improved handling characteristics and staining capabilities, increased solvent resistance, and a higher signal-to-noise ratio for enhanced sensitivities. The PolyScreen® membrane has a nominal pore size of 0.45 micron (μm) and is useful for blotting proteins > 10 kDa. It is an ideal substrate for immunodetection. PolyScreen® is compatible with standard blocking agents and detection protocols, including chemiluminescence. Because the membrane is composed of PVDF, it is also compatible with the harsh conditions used in protein sequencing and amino acid analysis. This insert describes how to perform electroblotting using a tank or semi-dry transfer system. It also provides steps on a new rapid immunodetection protocol and technical considerations for protein sequencing applications.

## HOW TO PREPARE AN SDS-PAGE GEL AND THE POLYSCREEN MEMBRANE

Before you start, have a sodium-dodecyl sulfate-polyacrylamide gel (SDS-PAGE gel) and a complex protein mixture available.

### Preparing the Gel

1. Resolve the protein mixture on the gel. The amount of protein loaded depends on the width of the wells, the dimensions of the gel, and the individual protein concentration in the sample. For optimum resolution, consider all these factors when loading.

CAUTION: Do not overload the gel or the bands may smear during the transfer. For example, 10 to 20 microgram (μg) of a complex protein mixture in each 8-millimeter (mm) lane resolves well on a mini-gel.

2. Open the gel cassette and lift off the gel with a clean razor blade. Then notch a corner of the gel. This provides orientation to the membrane after the transfer.

3. Prepare 100 milliliter (mL) of the appropriate buffer solution for your transfer method (Prepare 200 mL of buffer for larger gels). This chart describes the buffer compositions you can use:

| Transfer Method | Buffer | Composition |
|---|---|---|
| Tank transfer | Transfer buffer | 25 millimolar (mM) Tris base, 192 mM glycine, 10% methanol |
| Semi-dry transfer | Cathode buffer | 25 mM Tris base, 40 Mm 6-amino-n-capronic acid, 10% methanol, pH 9.4<br><br>NOTE: You can substitute glycine for 6-amino-n-caproic acid. |

4. Immerse the gel in the transfer buffer and allow it to equilibrate for 15 minutes.

**Preparing the Membrane**

Before you start, have a piece of the PolyScreen membrane, the prepared gel, methanol, and distilled water available.

1. Prepare 100 mL of transfer buffer for a tank transfer. For a semi-dry transfer, prepare 100 mL of anode buffer II. See the following chart for details:

| Transfer Method | Buffer | Composition |
|---|---|---|
| Tank transfer | Transfer buffer | 25 mM Tris base, 192 mM glycine, 20% methanol |
| Semi-dry transfer | Anode buffer II | 25 mM Tris, 10% methanol, pH 10.4 |

2. Cut a piece of the PolyScreen membrane to the dimensions of the gel. Notch or label one corner of the membrane to correspond to a corner of the gel.

3. Wet the membrane in 100% methanol for 15 seconds. Then transfer it to a container of distilled water for 2 minutes.

CAUTION: Use care when handling the membrane to prevent tearing. And do not leave any dry spots that can inhibit the transfer.

4. Equilibrate the membrane for at least 5 minutes in the transfer buffer for a tank transfer or in the anode buffer II solution for a semi-dry transfer.

NOTE: For alternative buffer systems, equilibrate the membrane in the buffer you choose.

5. Follow the steps in the next section for a tank transfer system. For a semi-dry system, see the "How to Perform a Semi-Dry Transfer" section instead.

## HOW TO PERFORM A TRANSFER WITH A TANK SYSTEM

To transfer the proteins from the gel using a tank transfer system, you need to:

- Prepare the buffer
- Assemble the transfer stack
- Transfer the protein
- Remove the blot

### A. Preparing the Buffer for a Tank Transfer Assembly

| Buffer | Composition | Amount |
|---|---|---|
| Tank transfer | 25 mM Tris base, 192 mM glycine, 10% methanol | A volume sufficient to fill the transfer tank. |

**Assembling the Transfer Stack for a Tank System**

Before you start, you need:

- One tank transfer system
- Two foam pads (for example, Scotch Brite® pads)
- Two sheets of Whatman® 3MM filter paper, cut to the dimension of the gel and soaked in tank transfer buffer for at least 30 seconds
- One glass pipette

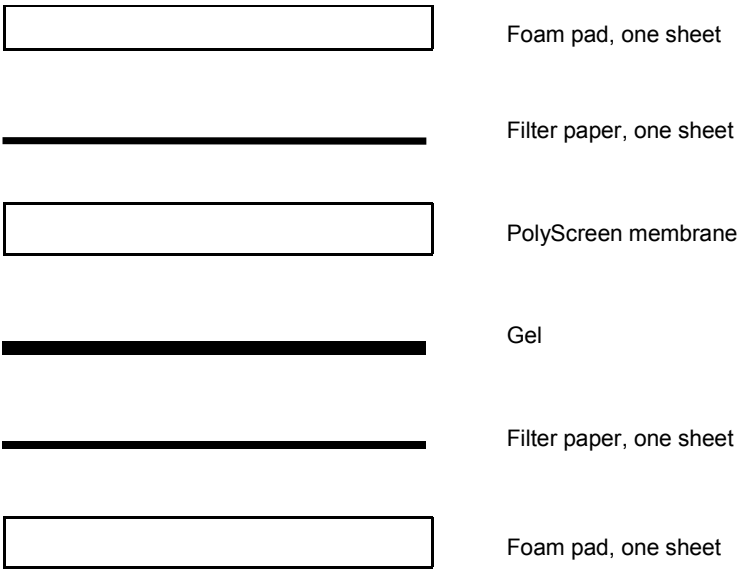
- Transfer buffer
- One prepared gel
- One prepared sheet of PolyScreen® membrane

1. Place a foam pad on one side of the cassette holder. Then place one sheet of filter paper on top of the pad.

CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a pipette over the surface of each layer in the stack. Do not apply excessive pressure or you may damage the membrane and gel.

2. Place the gel on top of the filter paper. Then put the sheet of the PolyScreen® membrane on top of the gel.

3. Place one sheet of filter paper on top of the stack. Then put a foam pad on top of the filter paper. The stack should look like this:




**Transferring the Protein Using a Tank System**

1. Close the tank transfer cassette holder. Then place it in the tank blotting apparatus so that the side of the cassette holder with the gel is facing the cathode (-). Add enough transfer buffer to the blotting apparatus to cover the cassette holder.

2. Insert the black cathode lead (-) into the cathode jack. Insert the red anode lead (+) into the anode jack. Then connect the anode lead and cathode lead to their corresponding power outputs.

3. Turn on the system for 1 to 2 hours at 6 to 8 V/cm inter-electrode distance to transfer the proteins to the membrane. You may need to cool the buffer during the transfer to prevent overheating.

**Removing the Blot from the Tank Transfer System**

1. Remove the cassette holder from the blotting apparatus. Open the cassette holder. Remove the foam pad and filter papers with forceps.

NOTE: Let the blot air-dry to improve the protein binding.

2. See the "How to Visualize the Proteins" section further in this document for details on staining. If you want to visualize the proteins with transillumination or perform a rapid immunodetection, see the "How to Dry the Blotted Membrane" section near the end of this document.

CAUTION: You must completely dry the PolyScreen membrane for specific lengths of time before performing transillumination or rapid immunodetection techniques. (See the chart in "How to Dry the Blotted membrane" section for time details). The drying enhances protein binding to the surface and reduces background noise.

## HOW TO PERFORM A TRANSFER WITH A SEMI-DRY SYSTEM

To transfer the proteins from the gel using a semi-dry system, you need to:

- Prepare the buffers
- Assemble the transfer stack for a single or multiple transfer
- Transfer the protein
- Remove the blot

**Preparing the Buffers for a Semi-Dry Transfer Assembly**

You need to prepare anode buffer I, anode buffer II, and cathode buffer solutions for a semi-dry solution. See this chart for details:

| Buffer | Composition | Amount |
|---|---|---|
| Anode buffer I | 0.3 M Tris, 10% methanol, pH 10.4 | 200 mL |
| Anode buffer II | 25 mM Tris, 10% methanol, pH 10.4 | 200 mL |
| Cathode buffer | 25 mM Tris base, 40 mM 6-amino-n-caproic acid, 10% methanol, pH 9.4<br><br>NOTE: You can substitute glycine for 6-amino-n-caproic acid. | 200 mL |

Continue to the next section to assemble a stack for a single transfer. For a multiple transfer, see "Assembling the Transfer Stack for a Semi-Dry System (Multiple Transfer)".

**Assembling the Transfer Stack for a Semi-Dry System (Single Transfer)**

To assemble the transfer stack, you need:

- One semi-dry blotter
- Six sheets of Whatman 3MM filter paper cut to the gel dimensions
- One sheet of prepared PolyScreen membrane
- One prepared gel
- Prepared anode and cathode buffers

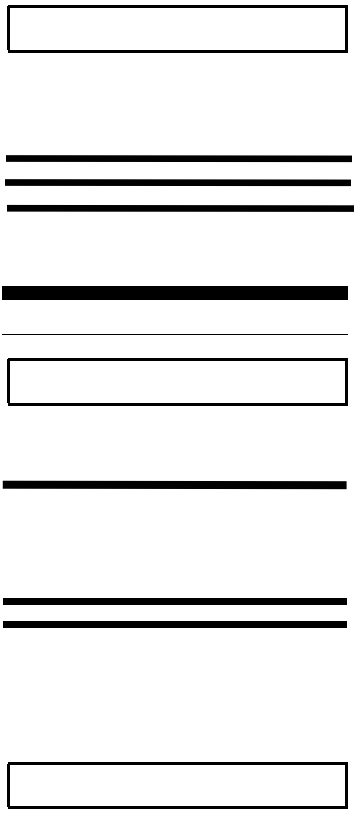
1. Place the anode electrode plate on a level bench top. Then wet two sheets of filter paper in the anode buffer I solution. Place them in the center of the graphite anode electrode plate.

CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a pipette over the surface of each layer of the stack. Do not apply excessive pressure or you may damage the membrane and gel.

2. Wet a sheet of filter paper in the anode buffer II solution. Place it on top of the first two sheets of filter paper. Place the PolyScreen membrane on top of the filter paper.

3. Place the gel on top of the membrane. Wet three pieces of filter paper in the cathode buffer solution. Place them on top of the gel.

4. Place the cathode plate cover on top of the assembled transfer stack. The stack should look like this:




CAUTION: Do not bump the cathode plate cover since it could disturb the alignment of the transfer stack and cause inaccurate results**.**

5. Continue to "Transferring the Protein Using a Semi-Dry System".

**Assembling a Transfer Stack for a Semi-Dry System (Multiple Transfer)**

This section describes how to assemble a transfer stack for a semi-dry system. You can transfer up to six gels in a single stack.

1. Locate the materials listed in the previous section "Assembling the Transfer Stack for a Semi-Dry System (Single Transfer)". You also need:

- Dialysis membrane, for multiple transfer stacks, cut to the dimensions of the gel and wet in distilled water.

NOTE: The dialysis membrane should have a molecular weight exclusion small enough to retain the lowest molecular weight protein in the gel.

- Whatman 3MM sheets cut to the dimensions of the gel.

2. Follow steps 1 and 2 from "Assembling the Transfer Stack for a Semi-Dry System (Single Transfer)".

3. Place the gel on top of the membrane.

4. Wet a sheet of filter paper, cut to the dimensions of the gel, in cathode buffer. Then place it on top of the gel.

NOTE: If you finished adding the last gel to the stack, place two pieces of filter paper, soaked in cathode buffer, on top of the stack. Then skip to step 9. If you need to add more gels to the stack, continue on to step 5.

5. Place a piece of dialysis membrane on top of the filter paper.

6. Wet a sheet of filter paper in anode buffer II and place it on top of the dialysis membrane.

7. Place a sheet of PolyScreen membrane on top of the filter paper.

8. Return to step 3 to build another transfer stack.

9. Place the cathode plate cover on top of the assembled transfer stack. Then continue to "Transferring the Protein Using a Semi-Dry System".

**Transferring the Protein Using a Semi-Dry System**

1. Insert the black cathode lead (-) into the cathode plate jack. Then insert the red anode lead (+) into the anode plate jack.

2. Connect the anode lead and cathode lead to their corresponding power supply outputs.

*3.* Turn on the power supply. Set the current and let it run for the appropriate time:

4.

| Current Density | Time Limit |
|---|---|
| 0.8 mA/cm$^2$ | 1 to 2 hours |
| 1.2 mA/cm$^2$ | 1 hour |
| 2.5 mA/cm$^2$ | 30 to 45 minutes |
| 4.0 mA/cm$^2$ | 10 to 30 minutes |

**Removing the Blot from the Semi-Dry System**

1. Turn off the power supply and disconnect the system leads when the transfer is complete.

2. Remove the cover. Then peel off and discard the filter papers.

NOTE: Graphite particles from the anode electrode plate occasionally appear on the filter paper. These particles do not affect the operation of the apparatus.

3. Peel off the gel.

4. Peel off the blotted membrane with a pair of forceps.

5. See the next section, “How to Visualize the Proteins”, for details on staining. If you want to visualize the proteins with transillumination or perform a rapid immunodetection, see “How to Dry the Blotted Membrane” further in this document.

CAUTION: You must completely dry the PolyScreen membrane for specific lengths of time before performing transillumination or rapid immunodetection techniques. (See the chart in “How to Dry the Blotted Membrane” section for time details). The drying enhances protein binding to the surface and reduces background noise.

## HOW TO VISUALIZE THE PROTEINS

When you complete the electrotransfer, you can stain the blot to assess the quality of the transfer. For example, you can use Coomassie® brilliant blue R, amido black, or Ponceau-S red stain.
If the stain could interfere with subsequent analysis, use transillumination to visualize the proteins. (To determine protein elution from the gel, stain it with Coomassie brilliant blue or a silver stain). See the following section for details on staining. Skip to “Using transillumination” for details on transillumination.

**Using Staining Methods**

This section describes how to stain with:

- Coomassie brilliant blue R
- Amido black
- Ponceau-S red

For details on visualizing proteins with transillumination, see the "How to Visualize with Transillumination" section.

CAUTION: If you plan to use the Coomassie brilliant blue R, amido black, or Ponceau-S red stain to visualize the proteins and the blot is dry, rewet it in 100% methanol before you stain it. This ensures accurate results.

**Coomassie Brilliant Blue R Stain**

To stain the blot with Coomassie blue, follow these steps.

CAUTION: This stain will interfere with rapid immunodetection. It is not reversible. Follow steps in the "Ponceau-S Red Stain" section or "How to Visualize with transillumination" for reversible methods.

1. Incubate the blot in a solution of 0.1% Coomassie brilliant blue R in 50% methanol, 7% acetic acid, for 2 minutes.
2. Destain the blot in 50% methanol, 7% acetic acid, for 10 minutes.
3. Incubate the blot in 90% methanol, 10% acetic acid for 10 minutes to completely destain the background.

**Amido Black Stain**

To stain the blot with amido black, follow these steps.

CAUTION: This stain will interfere with rapid immunodetection. It is not reversible. Follow the steps in the "Ponceau-S Red Stain" section or "How to Visualize with transillumination" for reversible techniques.

1. Incubate the blot in a solution of 0.1% amido black in 25% isopropanol, 10% acetic acid for 10 minutes.

2. Destain the blot in the same buffer without amido black for 5 to 10 minutes.

**Ponceau-S Red Stain**

To stain the blot with Ponceau-S red (reversible stain), follow these steps.

1. Stain the blot in a solution of 0.5% Ponceau-S red, 1% acetic acid, for 1 minute.

2. Destain the blot in distilled water to the desired contrast or wash the blot with 0.1 N NaOH to remove the stain completely.

**Using the Transillumination Method**

Transillumination is a nondesructive, reversible method of determining the presence of the appropriate protein pattern.
To perform transillumination on the blot, follow these steps:

1. Let the blot dry completely using one of the drying methods listed in the following section, "How to Dry the Blotted Membrane". Then return to this section.

2. Immerse the dry blot in 20% methanol for 2 minutes.

3. Place the blot on a light box and mask the areas around the blot with a sheet of black paper. The bands appear as clear areas against an opaque background.

NOTE: Detection sensitivity is comparable to Coomassie brilliant blue R.

## HOW TO DRY THE BLOTTED MEMBRANE

After removing the blotted membrane from a tank or semi-dry system. You should dry the membrane prior to conducting imunodetection procedures. Dehydrated proteins bind more strongly to the membrane, and this helps to prevent loss of target during subsequent steps. This

section lists four drying options. The length of time for each option varies. As the blot dries, it becomes opaque. You must wait the full length of drying time for all of the liquid to evaporate from within the membrane's pore structure.

To dry the membrane after blotting, select one of these methods:

| Drying Method Option | Required Drying Time |
|---|---|
| Soak the membrane in 100% methanol for 10 seconds to drive out the water. Then place the blot on a piece of filter paper. Wait for the methanol to evaporate. | 15 minutes |
| Place the blot in a vacuum chamber. (Secure the blot between two sheets of filter paper). | 30 minutes |
| Incubate the blot at 37°C | 1 hour |
| Place the blot on a lab bench to let it dry at room temperature. | 2 hours |

## PROTEIN DETECTION AND CHEMILUMINESCENCE

There are various ways to detect proteins bound to PolyScreen. First, there are direct, unspecific total protein stains such as colloidal gold, Coomassie brilliant blue, Ponceau-S or Amino black. The second method involves direct specific staining. This uses a probe ligand or specific antibody which is labeled with either radioactivity (e.g., $^{125}$I), a fluorescent compound (e.g., fluorescein) or an enzyme (e.g., horseradish peroxidase, alkaline phosphatase). The third and most common method uses an indirect immunolabel. This method uses a specific antibody as the first probe. This first probe binds directly to the target and is then detected by a second labeled antibody.

PerkinElmer Life Sciences offers a complete line of nonradioactive products to provide the researcher high sensitivity options for horseradish peroxidase (HRP) detection. The Western Lightning™ Chemiluminescence Reagent Plus is a convenient, fast detection product that provides hard-copy results on film or digital image on the *KODAK* Image Station. For researchers preferring chromogenic detection, PerkinElmer offers the low-toxicity HRP catalyzed substrate, 4CN Plus.

**Chemiluminescence: Detection and Reprobing**

The PerkinElmer brand Western Lightning Chemiluminescence Reagent Plus is a non-radioactive light-emitting system designed to detect proteins immobilized on a membrane. This method provides a sensitivity of 1-10 pg of protein and yields a permanent hard copy record on X-ray film or digital image with the *KODAK* Image Station. The unique substrate provides a high quantum yield light output over several hours, allowing for multiple exposures. Membranes can be stripped and reprobed if stored between uses.

The PerkinElmer Western Lightning Chemiluminescence Reagent Plus is based on an enhanced version of a chemiluminescence reaction in which the enzyme horseradish peroxidase (HRP) catalyzes light emission from the oxidation of luminol. Use of an enhancer increases the emission approximately 1,000-fold. Antibodies labeled with HRP are reacted directly or indirectly with the immobilized protein antigen. Following addition of the Chemiluminescence Reagent to the membrane, oxidative degradation of luminol occurs, resulting wavelength of 428 nm. This light is captured on the *KODAK* Image Station or *KODAK* X-OMAT Blue Autoradiography Film producing a permanent record of the results.

This is an outline of the chemiluminescence protocol. Consult the Western Lightning Chemiluminescence Reagent manual for complete protocols and troubleshooting.

**Membrane Preparation**

NOTE: All incubations require rocking or shaking. All steps are performed at room temperature.

1. Separate proteins by electrophoresis and transfer.
2. Block non-specific binding sites by incubating the membrane in 5% non-fat dry milk (or 1% BSA) in PBST for one hour.
3. Wash the membrane twice for 5 minutes with PBST.
4. Dilute the primary antibody in 1% BSA/PBST and incubate with the membrane for one hour.
5. Wash the membrane with PBST once for 15 minutes, and then four times for 5 minutes each.
6. Dilute the HRP-labeled second antibody in 1% BSA/PBST and incubate with the membrane for one hour.

7. Wash the membrane with PBST once for 15 minutes and then four times for 5 minutes each.

**Chemiluminescence Reagent Protocol**

1. Prepare the Chemiluminescence Reagent by mixing equal volumes of solution from Bottle 1 and Bottle 2.

2. Agitate the membrane for one minute with at least 0.125 mL of Chemiluminescence Reagent per $cm^2$.

3. Remove excess Chemiluminescence Reagent by draining or blotting and place the membrane in a plastic sheet protector.

4. Expose to *KODAK* X-OMAT Blue Autoradiography Film for 30 seconds. Develop the film and, if necessary, use the result to determine an optimum exposure.

**4C Plus (Cat. No. NEL300) Reagent Control**

4CN Plus is a modified, highly sensitive 4CN (5-chloro-1-naphthol) formulation designed for the chromogenic detection of horseradish peroxidase (HRP). The sensitivity of 4CN *Plus* is approximately 10X that of conventional formulations of 4CN and is approximately equal to or slightly better than diaminobenzidine (DAB). 4CN *Plus* is only available from PerkinElmer and patent applications are pending in the U.S. and in other countries. This product is intended to be used for research only and is not intended for resale.

The substrate solution is made by adding 200 µL of 4CN *Plus* reagent to 10 mL of a 1:10 dilution of 4CN *Plus* diluent. The membranes are then placed in the substrate solution and the color is allowed to develop for 30 min. at room temperature. The substrate reaction is terminated by placing the blots in distilled water. The membranes are then air-dried.

**Stripping and Reprobing**

Reprobing PolyScreen with the PerkinElmer Western Lightning Chemiluminescence Reagent is easy compared to chromogenic systems. Chromogenic systems deposit the colored substrate directly onto the membrane and color removal is difficult. In the chemiluminescence system, the oxidized substrate need not be removed. Methods for striping antibodies from membranes have been described for $^{125}$I-labeled proteins. These techniques can also be used for stripping antibodies used for chemiluminescence.

1. Wash membrane in PBST.

2. Incubate in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCI (pH 6.8), for 30 min. at 50°C.

3. Wash briefly in PBST.

4. (Optional) Incubate with chemiluminescence detection reagents to determine if the stripping procedure worked.

5. Block membrane, incubate in antibody solution, and detect with fresh chemiluminescence detection reagents.

## CONSIDERATIONS FOR PROTEIN SEQUENCING APPLICATIONS

Protein sequencing provides valuable amino acid sequence information; N-terminal sequencing is commonly performed by automated instruments designed for this purpose. The following sections provide information on the most common concerns about using the PolyScreen membrane for sequencing applications.

### Glycine

The glycine present in standard transfer buffers leads to a high background glycine peak in the first few cycles of protein sequencing and in amino acid analysis. To reduce the level of glycine, wet the membrane in 100% methanol and then wash extensively with distilled water. Alternatively, change the transfer buffer to 10 mM CAPS (3-[cyclohexylamino[-1-propanesulfonic acid), pH 11, 10% methanol. This buffer works well in tank transfer systems, but sometimes gives less efficient transfer in semi-dry systems.

### Coomassie Brilliant Blue Stain

Coomassie brilliant blue is compatible with automated protein sequencing chemistries. In general, if the protein band or spot can be visualized by Coomassie staining, there is enough protein for sequencing.

### Storage

For long-term storage, dry the blot completely. Then seal it in a plastic bag. Place the bag between two sheets of cardboard. Store the assembly at -20°C or colder to prevent oxidation. The cardboard protects the frozen membrane from breaks or cracks due to handling when removing the membrane from storage. Allow the membrane to thaw to ambient temperature before removing the cardboard.

### Protein Elution

For applications requiring protein elution from the membrane, visualize the proteins bands by transillumination or Coomassie staining in solutions free of acetic acid. The acid in staining solutions strengthens the interaction between proteins and the membrane, making elution more difficult.

### Amino Acid Analysis

For amino acid analysis, always analyze a piece of blank membrane from the same blot to account for any background signals.

## ORDERING INFORMATION

This section lists the catalogue numbers for PolyScreen.

**PolyScreen Catalogue Numbers**

| Membrane Dimensions (0.45 µM pore size) | Catalogue Number |
|---|---|
| 20 cm x 20 cm sheets (10 sheets) | NEF1000 |
| 26.5 cm x 3.75 m roll | NEF1002 |
| | |

## TECHNICAL ASSISTANCE

Call the office in your country to get additional product information, technical service, or order parts.

NOTE: To receive the current edition of our laboratory products catalogue (PerkinElmer), contact Technical Service at PerkinElmer office closest to you. You can also look us up on the Internet at www.perkinelmer.com/lifesciences.

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## PERKINELMER PRODUCTS FOR PROTEIN AND NUCLEIC ACID DETECTION

Improved PolyScreen Transfer Membrane

| | |
|---|---|
| NEF1000 | 10 sheets, 20 cm x 20 cm |
| NEF1002 | 1 roll, 26.5cm x 3.75 m |

Western Lightning Chemiluminescence Reagent

| | |
|---|---|
| NEL100 | For 1,000 $cm^2$ membrane |
| NEL101 | For 2,500 $cm^2$ membrane |
| NEL102 | For 5,000 $cm^2$ membrane |

Western Lightning Chemiluminescence Reagent Plus

| | |
|---|---|
| NEL103 | For 1,000 $cm^2$ membrane |
| NEL104 | For 2,500 $cm^2$ membrane |
| NEL105 | For 5,000 $cm^2$ membrane |
| NEL602 | CDP-*Star*™ |

4CN PLUS

| | |
|---|---|
| NEL300 | For 3,000 $cm^2$ of membrane, an enhanced chromogenic substrate and diluent for the detection of HRP |

*KODAK* X-OMAT Blue Autoradiography Film

| | |
|---|---|
| NEF595 | 100 Sheets of 14" x 17" |
| NEF596 | 100 Sheets of 8" x 10" |

All *KODAK*™ XAR and BioMAX Film available.

PROTEIN MARKERS

| | |
|---|---|
| NEL311 | Wide Range Color Markers |
| NEL312 | High Range Color Markers |
| NEL313 | Low Range Color Markers |
| NEL310 | Biotinylated Protein Molecular Weight Markers |


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United Kingdom: Tel: 0800 89 60 46 Fax: 0800 89 17 14 Switzerland: Tel: 0800 55 50 27
Fax: 0800 55 50 37 Italy: Tel: 800 79 03 10 Fax: 800 78 03 11 Sweden: Tel: 020 79 07 35
Fax: 020 79 07 37 Norway: Tel: 800 11 947 Fax: 800 11 948 Denmark: Tel: 80 88 3477
Fax: 80 88 34 78 Spain: Tel: 900 973 255 Fax: 900 973 256

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