BrightStar®-Plus Membranes

Positively Charged Nylon Membrane
Part Number AM10100, AM10102, AM10104

Applied Biosystems

A. Product Description

Part Number	Contents
AM10100	5 membranes: 15 cm x 15 cm
AM10102	Small roll: 30 cm x 45 cm
AM10104	Large roll: 30 cm x 3 m

Ambion® BrightStar®-Plus membranes are composed of a porous, nylon 66 matrix, derivatized with quaternary ammonium groups. The positively charged surface is maintained in the range of pH 3 to pH 10, which is favorable for strong ionic binding of nucleic acids. Our research has shown that this type of membrane gives the lowest detection limits in comparison to any other preparation. These membranes are suitable for:

- Northerns
- Southerns
- Dot Blots
- Non-radioisotopic detection systems

B. Dot Blots

The mass of material applied to a fixed area is critical, since there is a limit to the binding capacity of the membrane. Therefore target should not be mixed with carrier DNA or RNA since signal can be reduced by competition with the carrier.

Assemble the vacuum apparatus according to manufacturer's instructions with a pre-wetted membrane.

1. Prepare 100 µL samples of target nucleic acid in one of the 2 solutions shown below. (Add 1 µL of gel loading buffer if desired to help identify the location of spots on the membrane.)
   - RNA in 1 M NaCl/10 mM NaOH
   - DNA in 1 M NaCl/400 mM NaOH

2. Pull a vacuum through the membrane and apply the solution to the well.
3. Apply a second aliquot of the solution used to dilute the nucleic acid to wash the well.
4. Remove the membrane, label it, and immediately crosslink the nucleic acid to the membrane (see section E on page 5).

C. Downward Alkaline Transfer for Agarose Gels

IMPORTANT

This method should not be used with acrylamide gels; transfer acrylamide gels by electroblotting (see section D on page 4).

For optimum results with gels containing RNA, we recommend using NorthernMax® Transfer Buffer, P/N AM8672. Alternatively use one of the following transfer buffers:

- for RNA: 1 M NaCl/10 mM NaOH
- for DNA: 1 M NaCl/400 mM NaOH

1. Prepare blotting materials

a. Remove the unused gel above the wells
Use a razor blade or scalpel to cut through the wells. This prevents transfer buffer from flowing through the wells instead of through the gel during transfer.
To ensure proper orientation later on, cut a notch into the upper right hand corner of the gel and membrane.

b. Cut the BrightStar-Plus Membrane to the size of, or slightly larger than the gel
Handle the membrane only by the edges using gloved hands (preferably powder-free) or blunt forceps.

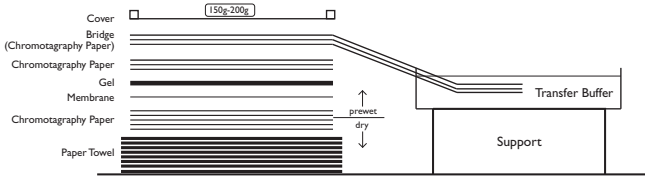
c. Cut 8 filter paper sheets to roughly the size of the gel.

d. Cut a 3 cm high stack of paper towels to about 1–2 cm wider than the agarose gel.

e. Put 0.5 mL transfer buffer per cm^2 of gel surface into a glass or plastic, flat bottomed container somewhat larger than the dimension of the agarose gel. This will be the reservoir for the transfer buffer.

f. Cut 3 filter paper bridges large enough to cover the gel and to reach into the reservoir.

2. Assemble the transfer materials as shown below



a. Arrange a stack of paper towels and blotting paper

i. Stack the pre-cut paper towels (~3 cm high) next to the transfer buffer reservoir.

ii. Put 3 dry pieces of filter paper on top of the paper towels.

iii. Wet 2 more pieces of filter paper in transfer buffer and add them to the top of the stack.

b. Position the membrane, then the gel on top of the blotting stack

i. Wet the BrightStar-Plus Membrane briefly in transfer buffer and place it on top of the stack of filter paper sheets. Be sure there are absolutely no bubbles trapped between the layers. Use a glass rod or Pasteur pipette to gently roll out any bubbles.

ii. Center the trimmed gel on the membrane, aligning the notches. Make sure the gel is upright i.e. in the same orientation as it was during electrophoresis, with the bottom of the gel in contact with the membrane. Roll out any bubbles.

c. Put the filter paper bridge in place

i. Wet 3 more pieces of filter paper in transfer buffer, and place them on the gel. Roll out any bubbles.

ii. Wet the 3 filter paper bridges in transfer buffer and place them on top of the stack, with one end in the reservoir.

d. Cover the stack with rigid light-weight plastic to prevent evaporation

We typically use the casting tray used to pour the gel. Place a small weight (150–200 g) on top of the stack to assure even contact of all the stack components.

IMPORTANT

Make sure the filter paper bridge is in contact with the transfer buffer in the reservoir, and that it does not touch the blotting stack below the gel. Laboratory film (e.g. Parafilm®) or plastic wrap can be placed around the edges of the gel to be sure that buffer must go through the gel.

3. Transfer for 15–20 min per mm of gel thickness or 4 hr

Transfer for a typical 6 mm thick gel should be 1.5–2 hours.

Do not exceed 4 hours because this can cause hydrolysis of small nucleic acids, reducing their hybridization signal.

4. Disassemble the transfer setup and crosslink the nucleic acid to the membrane

a. Remove the membrane with forceps and briefly (~10 sec) rinse in gel running buffer to remove salt and agarose. Briefly blot excess liquid, but do not dry the membrane, and immediately crosslink the nucleic acid to the membrane (see section E on page 5).

b. For samples stained with EtBr, verify transfer by examining the gel under UV light; there should be very little ethidium-stained material remaining in the gel at this step.

D. Electroblotting for Agarose or Acrylamide Gels

Electroblotting provides more force than passive elution. This is necessary for polyacrylamide gels and it may also be used with agarose gels.

Submerged, semi-dry, vacuum, or pressure blotters may be used. Follow the manufacturer's recommendations for transfer using their apparatus. Below we describe a method developed for a semi-dry transfer blotter:

1. Cut 6 pieces of filter paper to the size of the gel to be transferred.
2. Prepare 100 mL of 0.5X TBE electrophoresis buffer for wetting the papers.

3. Wet 2 filter papers and place them on the cathode plate of the electroblotter. Use a glass pipet to roll out any air bubbles.
4. Separate the glass plates and immobilize the gel onto a piece of filter paper. Lay the gel/filter paper on top of the wetted papers gel side up.
5. Cut the upper right-hand corner of the membrane for orientation. Wet the membrane and place on top of the gel. Roll out any air bubbles with a glass pipet.
6. Wet the last three filter papers and place on the stack. Take care to roll out any trapped air.
7. Wet the anode plate which will be in contact with the paper stack.
8. Place the anode plate on top and secure firmly.
9. Electrophorese for 30 min at 200 mA (constant current).
10. Disassemble and proceed with crosslinking.

E. Crosslinking RNA to BrightStar-Plus Membrane

There are 2 methods for immobilizing RNA on a membrane; both work equally well. For best results, dry the membrane after crosslinking. In humid climates it may be necessary to dry the membrane by applying low heat, such as that in a conventional oven.

UV crosslinking

UV crosslinking works by making a rigid covalent bond with the membrane, but it does destroy small parts of RNA. The membrane should be moist during crosslinking, since water does not absorb UV radiation. Most commercial crosslinkers have an automatic crosslink function which delivers a 120 mJ burst over 30 seconds. A hand-held lamp on the short wavelength setting will effect crosslinking in 1–2 minutes (see Ambion Technical Bulletin #169 for more information).

Baking

Baking works by heating the membrane to drive out all water from solubilizing the RNA. The only danger to baking is that the membrane can be damaged if the heat is not regulated to

keep it from rising above ~100°C. The oven does not need to be under vacuum for BrightStar-Plus Membrane because they are nylon.

F. Membrane Storage

Crosslinked membranes can be stored indefinitely at –20°C in a vessel that will protect them from physical damage (i.e. rolled up in a 50 mL tube).

G. Stripping Probe

Removing probe from membranes can be difficult, but is achieved by rigorous treatment with heat or base to disrupt hydrogen bonding between probe and target.

RNA probes on DNA or RNA targets

Alkaline procedures cannot be used here since high concentrations of base will hydrolyze RNA into 2'–3' cyclic NMPs. We suggest autoclaving the membrane in a bottle containing a 0.1% SDS solution for 15 minutes. Repeat if necessary.

DNA probes on DNA targets only

The same protocol used for RNA probe stripping can be used for DNA probes on DNA targets. Another option is alkaline denaturation; incubate the membrane in 400 mM NaOH for 30 minutes, then wash with 0.1% SDS for 15 minutes.

H. Safety Information

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

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