BrightStar®-Plus Positively Charged Nylon Membranes

Catalog #:	AM10100 – 5 membranes	15 cm x 15 cm
	AM10102 – Small roll	30 cm x 45 cm
	AM10104 – Large roll	30 cm x 3 m

Storage: Store at room temperature. This product is guaranteed for 6 months after date of shipment, if properly stored.

USER INFORMATION

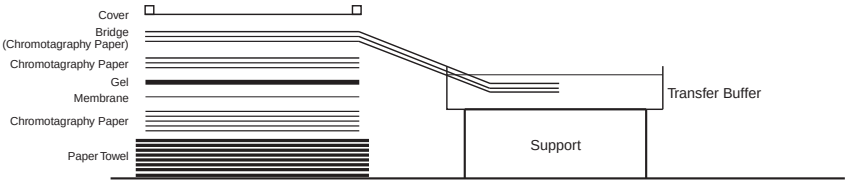
Ambion's BrightStar®-Plus membranes are composed of a porous, nylon 66 matrix, derivatized with quaternary ammonium groups. The positively charged surface is maintained in the range of pH 3 to pH 10, which is favorable for strong ionic binding of nucleic acids. Our research has shown that this type of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

- Northern blots
- Southern blots
- Dot blots
- Non-radioisotopic detection systems

NORTHERN AND SOUTHERN BLOTS

Passive transfer by denaturing agarose blotting (not meant for acrylamide gels)

The best low-tech method for agarose transfer is by a passive, slightly alkaline, downward elution (glyoxylated RNA **must** be transferred with an alkaline transfer system). This procedure, in comparison to upward transfer, is much faster and therefore results in tighter bands and higher signal (Note: Passive transfer from acrylamide gels is not very efficient, so we do not recommend it. Consider transfer by electroblotting instead.)



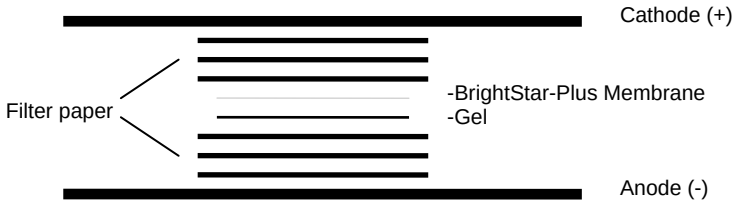
The composition of the transfer buffer is usually a 1 M NaCl/10 mM NaOH solution for RNA, and 1 M NaCl/400 mM NaOH for DNA. For optimum results, we recommend the use of Ambion's NorthernMax® Transfer buffer (Cat #AM8672) for RNA. The mildly alkaline conditions denature the RNA or DNA as it is deposited into the membrane. A brief protocol for assembly and transfer is as follows:

1. Remove the unused top portion of the gel by cutting through the wells with a razor blade. This prevents transfer buffer from flowing through the wells instead of the gel during transfer. To ensure proper orientation later, cut a notch into the upper right hand corner.
2. Place 0.5 mL of Transfer Buffer per cm^2 of gel surface into a glass or plastic, flat-bottomed container somewhat larger than the dimensions of the agarose gel. This will serve as the reservoir for the transfer buffer and will also be used to wet the blotting paper, membrane, and bridge.
3. Cut a stack of paper towels 1–2 cm wider than the agarose gel and stack tightly 2–3 cm high next to the transfer buffer reservoir.

4. Cut five filter paper sheets (e.g., Whatman 3 MM) the same size or slightly larger than the gel. These are stacked on top of the paper towels. The top-most 2 sheets should be wet in transfer buffer.
5. The membrane should be the same size or slightly larger than the gel. Notch the membrane to match the gel. When cutting the membrane to size, use a razor blade or scalpel. Handle the membrane only by the edges using gloved hands. Wet the membrane briefly in transfer buffer and place it on top of the stack of filter paper sheets. Be sure that there are no bubbles trapped between the layers. A glass rod or Pasteur pipette may be used to gently roll out any bubbles.
6. Lay the gel on top of the wet membrane, again smoothing out any bubbles.
7. Cut 3 sheets of filter paper to the same size as the gel, wet in the transfer buffer, and place on top of the gel. Again, be sure that there are no bubbles trapped between the layers.
8. Cut 3 filter paper bridges large enough to cover the area of the gel and to reach across into the transfer buffer reservoir. Wet these in transfer buffer and place on top of the stack. Make sure there are no bubbles trapped between any of the layers.
9. Cover the stack with the casting tray used to pour the gel. Otherwise, anything flat, rigid, and of similar area will work nicely. Place a 150–200 g object on top to lightly compress the stack. A small beaker filled with 100 mL of water also works well.
10. Make sure the filter paper bridge is in contact with the transfer buffer in the reservoir. There should be no path for the transfer buffer to follow from the reservoir to the dry filter paper and paper towels except through the gel. The most common place for “short circuiting” to occur is the area where the bridge enters the stack. If the bridge touches the filter paper or the paper towels underneath the gel, the flow of buffer will bypass the gel and reduce transfer efficiency drastically.
11. Allow the transfer to continue for ten minutes per millimeter of gel thickness. Do not let the transfer proceed for much more than 3 hours as this will begin to reduce hybridization signals.
12. Carefully disassemble the transfer apparatus. Remove the membrane with forceps and briefly rinse in 2 X SSC (0.3 M NaCl, 0.03 M sodium citrate) to remove salt and agarose.
13. The membrane should be treated to crosslink the RNA immediately after transfer and rinsing; see *Crosslinking Methods*, below.
14. The membrane may be stored wrapped in plastic wrap or in a 50 mL conical vial at 4°C until ready for analysis.

Electroblotting (for agarose or acrylamide gels)

Transfer from polyacrylamide gels requires more force than that offered by passive elution. The high-density matrix does not allow passive transfer in an efficient, quantitative, or reproducible manner. Thus, polyacrylamide gels should be “actively transferred” by electroblotting. This method may also be used with agarose gels. Electroblotting experiments at Ambion have shown that a ^{32}P/biotinylated RNA probe is transferred at 100% efficiency to Ambion’s BrightStar-Plus membranes, with no material left behind in the gel and none passing through the membrane:



Note that submerged, semi-dry, vacuum, or pressure blotters may be used. Follow the protocol recommended by the manufacturer for their apparatus. The method described here has been developed with the Owl semi-dry transfer blotter:

BrightStar®-Plus Membranes

1. Cut six pieces of filter paper to the size of the gel to be transferred.
2. Prepare 100 mL of 0.5 X TBE electrophoresis buffer for wetting the papers.
3. Wet two of the filter papers and place them on the cathode plate of the electroblotter. Use a glass pipet to roll out any air bubbles that may inhibit transfer.
4. After gel electrophoresis, 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 purposes, wet the BrightStar-Plus membrane, and place on top of the gel. Smooth out any air bubbles with a glass pipet.
6. Wet the last three filter papers and place on the stack. Take care to squeeze out any trapped air.
7. Wet the general area of the anode plate that will be in contact with the paper stack.
8. Place the anode plate on top and secure firmly, but not so tight that contact will occur outside of the stack.
9. Electrophorese for 30 min at 200 mA (constant current setting).
10. Disassemble and proceed with crosslinking discussed below.

DOT BLOTS

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

1. The vacuum apparatus should be assembled according to manufacturer's instructions with a pre-wetted membrane.
2. Prepare 100 µL-sized samples containing the target RNA in a 1 M NaCl/10 mM NaOH solution or DNA in a 1 M NaCl/400 mM NaOH solution. Spiking the solution with 1 µL of gel loading buffer gives the blots a blue tinge and helps in identifying the spots on the membrane for labeling purposes.
3. Pull a vacuum through the membrane and apply the solution to the well.
4. Apply a second aliquot of the buffer to wash the sides of the well.
5. Remove the membrane, label with a black permanent marker, and proceed to the crosslinking method of choice, discussed below.

CROSSLINKING METHODS

There are two methods for immobilizing nucleic acid on a membrane that both work equally well. UV crosslinking is one of the most popular and effective methods, using either a hand-held UV lamp at short wavelength, or a commercial crosslinking device. Alternatively, the membrane can be thermally baked in a conventional oven. For best results, the membrane must be dried either before or 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

Shortwave UV light causes the nitrogenous bases in nucleic acid to become highly reactive and to form covalent linkages to amine groups on the surface of the membrane. Although UV crosslinking does destroy small parts of the nucleic acid, it does not globally interfere with hybridization to a complementary probe. The membrane may be wet during crosslinking, as water does not absorb UV radiation. Most commercial crosslinkers have an automatic crosslink function that delivers a 120 milliJoule burst over a 30 second time period. A hand-held lamp on the short wavelength setting will cause crosslinking in 1–2 minutes. For more information see Ambion's Technical Bulletin 169 at www.ambion.com/techlib/tb/tb_169.html.

Baking

Baking works by heating the membrane to drive out all water from solubilizing the nucleic acid. A large component of nucleic acid is its hydrophobic nucleotide bases, which make hydrophobic contacts with aromatic groups on the membrane. This interaction is affected by heating in an oven at 80°C for 15 min.

Note: The membrane can be damaged the temperature rises over 100°C. The oven does not need to be under vacuum for nylon membranes.

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Storage

The membrane may be stored indefinitely once the transferred nucleic acids have been crosslinked. We recommend placing the membrane in a 50 mL screw-top vial and storing in a –20°C freezer. The membrane is ready to be used at a later date and does not require special handling after thawing.

STRIPPING PROBE

The removal of probe from membranes can prove to 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 here. Another option is alkaline denaturation. The membrane is incubated with 400mM NaOH for 30 minutes, then washed with 0.1% SDS for 15 minutes.

Ambion's Strip-EZ® technology provides an alternative to harsh stripping protocols via synthesis of probes with modifications that facilitate their degradation following hybridization and detection. For more information visit Ambion's Strip-EZ Resource page at:
www.ambion.com/techlib/resources/stripez/index.html

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