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ProductInformation

BioBond-PlusÔ and BioBondÔ Nylon membranes

Catalog Numbers **See Table**
Store at Room Temperature

TECHNICAL BULLETIN

Size	Quantity	BioBond-Plus Positively Charged Membrane	BioBond Neutral Membrane
11.9 cm x 7.8 cm	20 & 50 sheets	N5281	N1406
12.5 cm x 8 cm	20 & 50 sheets	N5781	N4031
15 cm x 20 cm	10 sheets	N5406	N3656
20 cm x 20 cm	2 & 10 sheets	N5531	N3781
30 cm x 60 cm	5 sheets	N5656	N3906
82 mm	50 discs		N4156
132 mm	50 discs	N6156	N4281
137 mm	50 discs	N8656	N4406
20 cm x 3.5 m	1 roll	N5031	N1281
20 cm x 12 m	1 roll	N5156	N8531
30 cm x 3.0 m	1 roll	N4781	
30 cm x 3.5 m	1 roll		N1031
30 cm x 12 m	1 roll	N4906	N1156

Immobilization of nucleic acids on solid supports is an integral part of any laboratory using molecular biology techniques. The choice of solid support is pivotal in the design of any experiment involving nucleic acid hybridization. BioBond Plus and BioBond nylon membranes provide quality results in any of the common blotting techniques – Southern, Northern, and dot/slot blots. Both positively charged and neutral membranes perform well in radioactive and non-radioactive detection methods. The neutral membranes are recommended for use in chemiluminescent detection.

Reagents required but not provided

- Agarose, Catalog Numbers A5093, A9539
- Alkaline Southern Transfer Solution; 0.4 M NaOH, 1.5 M NaCl
- Blot Stain Blue, Catalog No. B1177
- Denaturation Solution For Neutral Southern Transfer, Catalog No. N1531
- Depurination Solution for Neutral Southern Transfer; 0.2 N HCl
- Ethidium bromide solution, 0.5 mg/ml, Catalog No. E1385
- Formamide, deionized, Catalog No. F9037
- Mild Alkaline Northern Transfer Solution; 7.5 mM NaOH
- Mild Alkaline Northern Wash Solution; 2× SSC, 0.1% SDS
- Neutral Northern Transfer Solution; 10× SSC (Saline-sodium citrate buffer)
- Neutral Southern Transfer Solution; 10× SSC
- Neutralizing Solution for Alkaline Southern Transfer; 0.5 M Tris-HCl, 1.5 M NaCl
- Neutralizing Solution For Neutral Southern Transfer, Catalog No. N1532
- Proteinase K, Catalog Numbers P2308, P4850
- Quickdraw™ extra thick blotting paper, Catalog Numbers P7176, P8171, P7921, P8549, P6803, P8046, P7796, P6928
- RNA Sample Loading Buffer, Catalog No. R4268
- SDS, 10% solution, Catalog No. L4522
- Sodium hydroxide solution 5 M, Catalog No. S8263
- SSC Buffer 20X concentrate, Catalog No. S6639
- Water, Molecular Biology Grade, Catalog No. W4502

Procedures

Outlined on the following pages are standard protocols for the transfer of nucleic acids to BioBond nylon membranes. In addition to standard capillary transfer, BioBond membranes also perform well when used according to manufacturer's instructions for vacuum, pressure or electrophoretic blotting apparatuses and for dot or slot blot manifolds.

Standard Transfer of DNA (Southern Blotting)

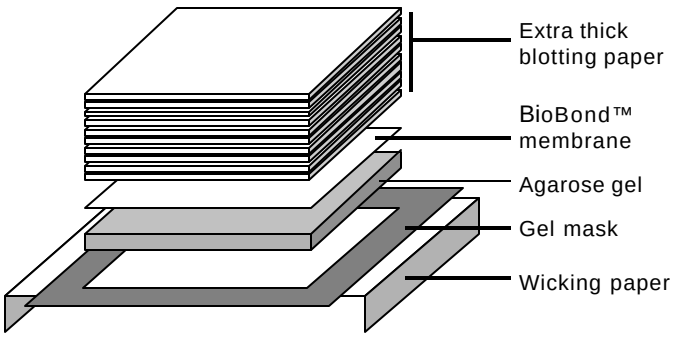
1. Subject DNA to electrophoresis on an agarose gel containing the appropriate percentage of agarose to resolve the bands of interest. For large fragments (0.8-10+ kb) use 0.7% agarose, for medium fragments (0.5-7 kb) use 1.0% agarose, and for small fragments (0.2-3 kb) use 1.5% agarose.

   Notes

   a. For most efficient transfers, always use the minimum agarose concentration necessary to resolve the bands of interest, and cast the gel not more than 7 mm thick.

   b. Steps 2-4 should be performed at room temperature with gentle agitation.
2. Depurination: If the fragments of interest are larger than 15 kb, the DNA should be nicked by depurination prior to transfer. To depurinate the DNA, soak the gel in several gel volumes of Depurination Solution For Neutral Southern Transfer for 10 minutes.
3. Denaturation: Briefly rinse the gel with deionized water to remove any residual running buffer or Depurination Solution. Denature the DNA by soaking the gel for 30 minutes in several gel volumes of Denaturation Solution For Neutral Southern Transfer.
4. Neutralization: Briefly rinse the gel with deionized water to remove any residual Denaturation Solution. Neutralize by soaking the gel for 30 minutes in several gel volumes of Neutralizing Solution for Neutral Southern Transfer.
5. While the gel is neutralizing, prepare membrane and filter paper for transfer. Prepare a blotting wick by cutting a piece of medium thickness blotting paper slightly wider and about 5 cm longer than the gel. Wrap the wicking paper around a piece of Plexiglass or a gel running tray that will serve as a support for gel and blotting paper. Place the wick and support in a tray containing a sufficient volume of Neutral Southern Transfer Solution for the entire transfer process. Make sure that both ends of the wick are in good contact with the transfer solution and that the level of the solution is below the top of the support. Allow the wick to wet completely and remove any trapped air bubbles by rolling a disposable pipet over the surface. Cut BioBond membrane and 10 pieces of Quickdraw blotting paper to the size of the gel. Pre-wet the membrane and one piece of Quickdraw in Transfer Solution.
6. Assemble the transfer apparatus for a standard upward capillary transfer as follows (see Figure 1). At each step carefully remove air bubbles by rolling a disposable pipet over the surface.
   - Place the neutralized gel on the thoroughly wetted wicking paper.
   - Cover the exposed areas of the wick with strips of parafilm or plastic wrap to prevent transfer buffer from bypassing the gel during the transfer process.
   - Place the pre-wetted membrane on top of the gel and mark for orientation with a pencil.
   - Carefully position the pre-wetted piece of Quickdraw blotting paper on top of the membrane.
   - Top this with the remaining 9 dry sheets of Quickdraw extra thick blotting paper.
   - Place a glass or plastic plate on top of the stack and top with 200-500 g of weight.
     Note: Excessive weight will cause compression of the gel resulting in inefficient transfer.
   - Allow transfer to proceed for 2 hours. When using Quickdraw blotting paper, two hours is sufficient for complete transfer. If required, the transfer can be allowed to proceed overnight with no reduction in transfer efficiency. Paper towels may be used in place of Quickdraw blotting paper; in this case allow the transfer process to proceed for 6-18 hours.

Figure 1



7. After transfer is complete, remove all blotting paper leaving the membrane on top of the gel. Mark the position of the wells using a soft lead pencil. Carefully lift membrane from gel and rinse briefly in 2X SSC to remove any agarose that may be stuck to the membrane.
8. Transfer the membrane to fresh pieces of blotting paper or dry paper towels and allow to air dry several minutes. To permanently affix the DNA to the membrane, bake at 80°C for 30 minutes and/or irradiate the membrane (DNA side toward the light source) with 120 mjoules of 254 nm ultraviolet light.
9. The membranes can be stored at room temperature between clean pieces of blotting paper until needed.
10. To assess the efficiency of transfer, the gel may be stained in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualized with ultraviolet light. In addition, the nucleic acids can be visualized on the membrane by staining with Blot Stain Blue.

Alkaline Transfer of DNA

1. Subject DNA to electrophoresis on an agarose gel containing the appropriate percentage of agarose to resolve the bands of interest. For large fragments (0.8-10+ kb) use 0.7% agarose, for medium fragments (0.5-7 kb) use 1.0% agarose, and for small fragments (0.2-3 kb) use 1.5% agarose.
   Note: Steps 2 and 3 should be performed at room temperature with gentle agitation.
2. Depurination: If the fragments of interest are larger than 15 kb, the DNA should be nicked by depurination prior to transfer. To depurinate the DNA, soak the gel in several gel volumes of Depurination Solution For Neutral Southern Transfer for 10 minutes at room temperature
3. Denaturation: Briefly rinse the gel with deionized water to remove any residual running buffer or Depurination Solution. Denature the DNA by soaking the gel for 30 minutes in several gel volumes of Alkaline Southern Transfer Solution
4. Transfer the DNA by capillary action as described in steps 5 and 6 of the standard protocol outlined above substituting Alkaline Southern Transfer Solution for the Neutral Southern Transfer Solution.
5. After transfer is complete, remove all blotting paper leaving the membrane on top of the gel. Mark the position of the wells using a soft lead pencil. Carefully lift membrane from gel and incubate 15 minutes with gentle agitation in Neutralizing Solution for Alkaline Southern Transfer to neutralize membrane and remove any agarose that may be stuck to the membrane.
6. Transfer the membrane to fresh pieces of blotting paper or dry paper towels and allow to air dry several minutes. To permanently affix the DNA to the membrane, bake at 80°C for 30 minutes and/or irradiate the membrane (DNA side toward the light source) with 120 mjoules of 254 nm ultraviolet light.
7. The membranes can be stored at room temperature between clean pieces of blotting paper until needed.
8. To assess the efficiency of transfer, the gel may be stained in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualized with ultraviolet light. In addition, the nucleic acids can be visualized on the membrane by staining with Blot Stain Blue.

Standard Transfer of RNA (Northern Blotting)

Note: Always utilize standard procedures to prevent RNase contamination throughout this protocol.

1. Subject RNA to electrophoresis in formaldehyde or glyoxal/DMSO gels according to published procedures. Sigma has found that addition of formaldehyde to MOPS buffered gels is not required when RNA has been denatured 10 minutes at 65°C in RNA Sample Loading Buffer prior to loading.
2. For RNA gels containing formaldehyde, wash the gel twice for 15 minutes in several gel volumes of RNase free water to remove residual formaldehyde. Glyoxal/DMSO gels do not require any pretreatment.
3. Transfer the RNA by capillary action as described in steps 5 and 6 of the standard protocol outlined above substituting either Neutral Northern Transfer Solution or Mild Alkaline Northern Transfer Solution for the Neutral Southern Transfer Solution.
   Note: Allow transfer to proceed for 2 hours. If required, the transfer can be allowed to proceed up to 6 hours with no reduction in transfer efficiency.
4. After transfer is complete, remove all blotting paper leaving the membrane on top of the gel. Mark the position of the wells using a soft lead pencil. Carefully lift membrane from gel and rinse briefly in 2X SSC buffer (Catalog No. S6639, 20X SSC, diluted 1:10 with water) to remove any agarose that may be stuck to the membrane.
5. Transfer the membrane to fresh pieces of blotting paper or dry paper towels and allow to air dry several minutes. To permanently affix the RNA to the membrane, bake at 80°C for 30 minutes and/or irradiate the membrane (RNA side toward the light source) with 120 mjoules of 254 nm ultraviolet light.
6. The membranes can be stored at room temperature between clean pieces of blotting paper until needed.
7. To assess the efficiency of transfer, the gel may be stained in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualized with ultraviolet light. In addition, the nucleic acids can be visualized on the membrane by staining with Blot Stain Blue, eliminating the need for ethidium bromide.

Colony or Plaque Lifts

1. Cool plates containing colonies or plaques at 4°C for at least 30 minutes.
2. Carefully place a BioBond membrane disc on the surface of the plate and label asymmetrically by piercing membrane and agar with a red-hot needle. Allow the membrane to wet completely.
3. Carefully remove the membrane from the plate. Place the membrane with the colony or plaque side up on filter papers soaked with Denaturation Solution for Neutral Southern Transfer and incubate for 5 minutes at room temperature.
4. Remove membrane from Denaturation Solution for Neutral Southern Transfer, blot excess liquid off the membrane, and place on filter papers soaked with Neutralizing Solution for Neutral Southern Transfer. Allow membranes to neutralize for 5 minutes at room temperature.
5. Transfer the membrane to fresh pieces of blotting paper and allow to air dry several minutes. To permanently affix the nucleic acids to the membrane, bake at 80°C for 30 minutes and/or irradiate the membrane (colony/plaque side toward the light source) with 120 mjoules of 254 nm ultraviolet light.
6. The membranes can be stored at room temperature between clean pieces of blotting paper until needed.
7. Optional: To improve signal/noise ratio, protein left from the colonies or plaques may be removed prior to hybridization by incubating the membranes 6 hours to overnight at 37°C in a solution containing 100 µg/ml proteinase K in 50 mM Tris-HCl, pH 7.6, 0.1% SDS, 50 mM NaCl. Rinse membrane in 2X SSC for at least 5 minutes prior to hybridization.

Stripping and Reproving

If blots are to be stripped and reproved, it is imperative that they are never allowed to dry out after being exposed to probe in hybridizations. Upon drying, the probe may become irreversibly bound to the membrane. Several procedures for stripping labeled probes from blots are outlined below:

Formamide Stripping of DNA Blots

1. Incubate membranes with constant agitation in a large volume (>200 ml) of 55% deionized formamide, 2X SSC, 1% SDS for 60 minutes at 65°C.
2. Rinse membranes 15 minutes at room temperature in 0.1X SSC, 0.1% SDS.
3. Confirm complete removal of probe by re-exposing the membrane to film.
4. Membranes can be used immediately for hybridizations or stored at -20°C wrapped in plastic.

Formamide Stripping of RNA Blots

1. Incubate membranes with constant agitation in a large volume (>200 ml) of 75% deionized formamide, 10 mM sodium phosphate, pH 7.2, for 60 minutes at 65°C.
2. Rinse membranes 15 minutes at room temperature in 0.1X SSC, 0.1% SDS.
3. Confirm complete removal of probe by re-exposing the membrane to film.
4. Membranes can be used immediately for hybridizations or stored at -20°C wrapped in plastic.

Stripping RNA or DNA Blots by Boiling in SDS

1. Bring approx. 400 ml of 0.1% SDS to a boil.
2. Pour approx. 200 ml of boiling 0.1% SDS solution on the blots and agitate for 5 minutes.
3. Replace SDS solution with remaining boiling 0.1% SDS solution and agitate blots until they come to room temperature.
4. Confirm complete removal of probe by re-exposing the membrane to film.
5. Membranes can be used immediately for hybridizations or stored at -20°C wrapped in plastic.

Alkaline Stripping of DNA Blots

Note: RNA blots should not be stripped in this manner.

1. Incubate membranes with constant agitation in a large volume (>200 ml) of 0.4 N NaOH for 30 minutes at 45°C.
2. Rinse membranes 15 minutes at room temperature in 0.1X SSC, 0.1% SDS.
3. Confirm complete removal of probe by re-exposing the membrane to film.
4. Membranes can be used immediately for hybridizations or stored at -20°C wrapped in plastic.

References

1. Maniatis, T., *et al*., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. (1989)
2. Ausubel, F.M., *et al*., Short Protocols in Molecular Biology, John Wiley and Sons Inc., USA,. (1995)

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