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ProductInformation

ALKALINE SOUTHERN BREEZE™ BLOTTING KITS

Product Numbers **ASBRZ-1A, ASBRZ-2A, ASBRZ-1B, ASBRZ-2B**
Store at room temperature
Technical Bulletin MB-635
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TECHNICAL BULLETIN

	With BioBond™ Plus positively charged membrane	With BioBond™ neutral membrane
Alkaline Southern Breeze™ Blotting Kit for 7x10 cm gels	**ASBRZ-1B**	**ASBRZ-1A**
Alkaline Southern Breeze™ Blotting Kit for 11x14 cm gels	**ASBRZ-2B**	**ASBRZ-2A**

Product Description

Southern blotting of deoxyribonucleic acids on solid supports is an integral part of any laboratory using molecular biology techniques. Sigma's Alkaline Southern Breeze™ blotting kits provide standard reagents for transfer in convenient single use packages. Additionally, the kit components have been optimized to provide consistent efficient transfer in the shortest possible time. Each complete kit includes all of the solutions for the pretreatment and transfer of deoxyribonucleic acid from agarose gels as well as precut BioBond™ nylon membranes and precut wicking and blotting papers. In addition, these kits also provide convenience items such as gel masking frames to prevent "short circuits" during capillary transfer. Although BioBond positively charged or neutral membranes included in these kits will perform well in both radioactive and non-radioactive detection methods, Sigma recommends the use of the neutral membrane for superior results with chemiluminescent detection systems.

Reagents

Sufficient for 10 alkaline Southern blots

- Southern Breeze blotting packs (BioBond membrane, blotting paper, wicking paper) — 10 packs
- Gel mask — 1 each
- 5X Depurination Solution, Product No. D0437 — 500 ml or 2 x 500 ml
- 5X Denaturation Solution, Product No. D0312 — 500 ml or 2 x 500 ml
- 5X Alkaline Neutralization Solution, Product No. R8148 — 500 ml or 2 x 500 ml
- 5X Alkaline Transfer Solution, Product No. T1440 — 500 ml or 2 x 500 ml

Precautions and Disclaimer

Sigma's Alkaline Southern Breeze Blotting Kits are for laboratory use only. Not for drug, household or other uses.

Storage/Stability

Store all materials at room temperature. Solutions have a shelf life of 1 year upon receipt. Blotting materials may be stored indefinitely.

Procedure

Note: Indicated volumes are for 7x10 cm gels and smaller. Volumes for larger gels are indicated in parentheses.

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

2. While gel is running, make dilutions of each of the transfer solutions to working concentration by combining 50 ml (100 ml) of each 5X solution with 200 ml (400 ml) of deionized water.

Steps 3 and 4 should be performed at room temperature with gentle agitation.

3. 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 with gentle agitation in 250 ml (500 ml) of 1X Depurination Solution for 30 minutes.

4. Denaturation: Briefly rinse the gel with deionized water to remove any residual running solution or depurination solution. Denature the DNA by soaking the gel for 30 minutes in 250 ml (500 ml) of 1X Denaturation Solution.

5. While the gel is denaturing, prepare membrane and filter paper for transfer. Remove the membrane, the 10 pieces of extra thick blotting paper and wicking paper from the protective sealed bag. 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 250 ml (500 ml) of 1X Alkaline Transfer Solution. 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.

6. Assemble the transfer apparatus as follows (see Figure 1). At each step carefully remove air bubbles by rolling a disposable pipet over the surface.

   - Center the gel mask on the thoroughly wetted wicking paper.
   - Carefully align the denatured gel on the gel mask so the mask overlaps the edges of the gel slightly.
   - Pre-wet the membrane and <u>one piece</u> of the blotting paper in the denaturation solution from which the gel was removed.
   - Place the pre-wetted membrane on top of the gel and mark for orientation with a pencil.
   - Carefully position the pre-wetted piece of extra thick blotting paper on top of the membrane.
   - Top this with the remaining 9 dry sheets of extra thick blotting paper.
   - Place a glass or plastic plate on top of the stack and top with ~100 grams of weight.

   Note: Excessive weight will cause compression of the gel resulting in inefficient transfer.

   - Allow transfer to proceed for 2 hours. If required, the transfer can be allowed to proceed overnight with no reduction in transfer efficiency.

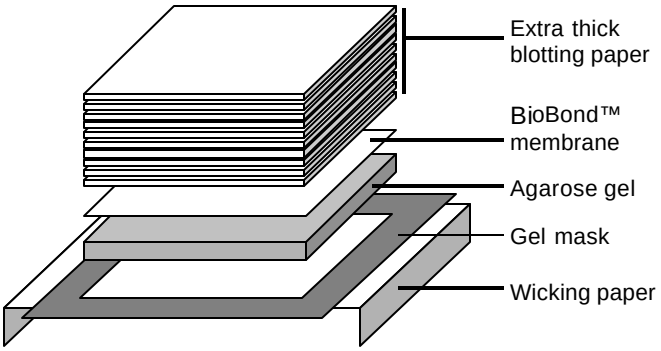
7. After transfer is complete, remove all blotting paper leaving the membrane on top of the gel. Mark the position of the wells using a soft lead pencil. Carefully lift membrane from gel and neutralize by incubating membrane 5 minutes in 250 ml (500 ml) of 1X Neutralization Solution with gentle agitation.

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 restained in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualized with ultraviolet light. To avoid using ethidium bromide, deoxyribonucleic acid can be visualized on the membrane by staining with Blot Stain Blue.

Figure 1



Stripping and Reprobing of BioBond Nylon Membranes

If membranes are to be stripped and reprobed, 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. Three procedures for stripping labeled probes from membranes 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.

Stripping of DNA Blots Using 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 membranes and agitate for 5 minutes.
3. Replace SDS solution with remaining boiling 0.1% SDS solution and agitate membranes 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

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.

General References

Maniatis, T., *et al*., (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, NY

Ausubel, F.M., *et al*., (1995) Short Protocols in Molecular Biology. John Wiley and Sons Inc., USA

Troubleshooting Guide

Problem:	Cause:	Solution:
High Background	Non-specific binding of probe to target nucleic acids	Add sheared, denatured salmon testis DNA (Product No. D7656) to a final concentration of 100 µg/ml in prehybridization and hybridization solutions
	Wash conditions not sufficiently stringent	Add an ultra-high stringency wash step (0.1X SSC, 0.1% SDS). Wash for 20 minutes at hybridization temperature. Increase the temperature of the hybridization and/or washes
	Exposure to film was too long	Shorten the exposure time to film.
	Concentration of enzyme conjugate in non-radioactive detection is too high	Dilute the enzyme conjugate further. The specific dilution required for optimal signal to noise must be determined empirically.
Weak/Absent Signal	Probe was not labeled efficiently	Check that the specific activity of radiolabeled probes is >5 x10^8 cpm/µg. For non-radioactive probes, check the incorporation of hapten by spotting and detecting serial dilutions of probe in direct comparison to a known standard. If probes are not labeled well enough, remake and confirm adequate incorporation rates.
	Target nucleic acids are not present, have been degraded, or are too low for detection.	Run agarose gel electrophoresis to confirm nucleic acids are not degraded. Load more target nucleic acids for blotting. For Southern blots, up to 10 µg DNA can be loaded per lane.
	Non-radioactive detection system is not working properly.	Confirm the enzyme/antibody conjugate is functioning properly by spotting and detecting the labeled probe on nylon membrane. If the enzyme/antibody conjugate is functional, check the chemiluminescent substrate by spotting the enzyme/antibody conjugate on a membrane and detecting with the substrate in question.

Related Products

Southern Breeze™ Blotting Kits, Product No. SBRZ-1A, SBRZ-1B, SBRZ-2A, SBRZ-2B
Northern Breeze™ Blotting Kits, Product No. NBRZ-1A, NBRZ-2A, NBRZ-1B, NBRZ-2B
Blot Stain Blue, Product No. B1177
Agarose, Product No. A9539
TBE Buffer, Product No. T4415, T6400, T9525, T7527
TAE Buffer, Product No. T9650, T6025, T8280, T4038
BlueView™ TAE, Product No. T8935
BlueView™ TBE, Product No. T9060
0.5 mg/ml Ethidium bromide, Product No. E1385
Sodium hydroxide, Product No. S8263
5 M Sodium chloride, Product No. S5150
Sodium phosphate, Product No. S3264, S3139
20X SSC, Product No. S6639, S8015, S0902
Deionized formamide, Product No. F9037
PerfectHyb™ PLUS hybridization buffer, Product No. H7033
All-in-One Nick Translation Labeling Mix –dCTP, Product No. N9155, N8530
All-in-One Nick Translation Labeling Mix –dATP, Product No. N8405, N9280
All-in-One Random Prime Labeling Mix –dCTP, Product No. R7522, R9647
All-in-One Random Prime Labeling Mix –dATP, Product No. R7022, R9522
ChromaTrack, Product No. C8468