NORTHERN BREEZE™ BLOTTING KITS

ProductInformation

Product No. **NBRZ-1A, NBRZ-2A, NBRZ-1B, NBRZ-2B**
Store at room temperature
Bulletin No.MB-640
May 2000

TECHNICAL BULLETIN

	With BioBond™ Plus positively charged membrane	With BioBond™ neutral membrane
Northern Breeze™ Blotting Kit for 7x10cm gels	**NBRZ-1B**	**NBRZ-1A**
Northern Breeze™ Blotting Kit for 11x14cm gels	**NBRZ-2B**	**NBRZ-2A**

Product Description

Northern blotting of ribonucleic acid onto solid support is an integral part of any laboratory using molecular biology techniques. Sigma's Northern Breeze™ blotting kits provide standard reagents for several types of transfer techniques 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 ribonucleic 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 both perform well in 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 Northern blots

- Northern Breeze Blotting Packs (BioBond membrane, blotting paper, wicking paper) — 10 packs
- Gel mask — 1 each
- 5X Gel Equilibration Solution, Product No. E2650 — 500 ml or 2 x 500 ml
- 5X Northern Transfer Solution, Product No. T1190 — 500 ml or 2 x 500 ml
- 5X Northern Rinsing Solution, Product No. R8023 — 500 ml or 2 x 500 ml

Precautions and Disclaimer

Sigma's Northern 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 at least 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 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 formaldehyde loading dye (Product No. R4268) prior to loading.
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.
3. Equilibration: To remove formaldehyde and equilibrate the gel for transfer, soak the gel for 30 minutes at room temperature with gentle agitation in 250 ml (500 ml) of 1X Gel Equilibration Solution.

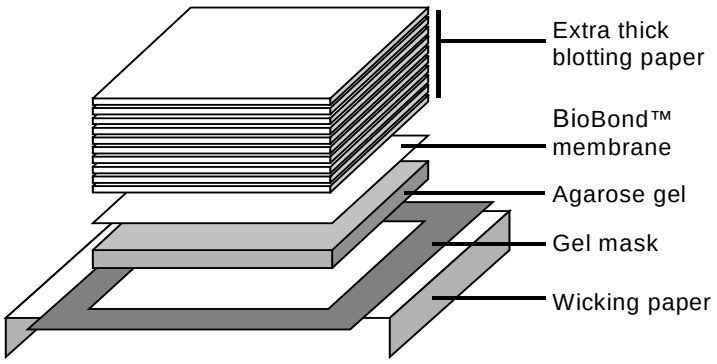
4. While the gel is equilibrating, prepare membrane and filter paper for transfer. Remove the membrane, the 10 pieces of extra thick blotting paper and wicking paper from the protective sealed bag. Wrap the wicking paper around a piece of plexiglass or a gel running tray that will serve as a support for gel and blotting paper. Place the wick and support in a tray containing 200 ml (400 ml) of 1X Northern 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.
5. Assemble the transfer apparatus as follows (see Figure 1). At each step carefully remove air bubbles by rolling a clean test tube or pipet over the surface.
    - Center the gel mask on the thoroughly wetted wicking paper.
    - Carefully align the equilibrated 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 equilibration 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 up to 6 hours with no reduction in transfer efficiency. Overnight transfers are not recommended.

6. 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 10 minutes with gentle agitation in 250 ml (500 ml) of 1X Northern Rinsing Solution to remove any agarose that may be stuck to the membrane.
7. 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.
8. The membranes can be stored at room temperature between clean pieces of blotting paper until needed.
9. 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. In addition, the nucleic acids can instead 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. Two procedures for stripping labeled probes from membranes are outlined below:

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 of RNA Blots by 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 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.

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

Results

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 Northern blots, up to 30 µg of total RNA 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
Alkaline Southern Breeze™ Blotting Kits, Product No. ASBRZ-1A, ASBRZ-1B, ASBRZ-2A, ASBRZ-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
MOPS-EDTA-sodium acetate buffer, Product No. M5755
RNA sample running buffer, Product No. R4268
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