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Product Information

Denaturation Solution for Neutral Southern Transfer

Catalog Number **N1531**

Neutralizing Solution for Neutral Southern Transfer

Catalog Number **N1532**

Store at Room Temperature

Product Description

Blotting of nucleic acids on solid supports is an integral part of any laboratory using molecular biology techniques. Sigma has developed a series of solutions that aid in ease and efficiency of transfer of nucleic acids from agarose gels to membranes.

The Denaturation Solution (denaturing solution) is a strong base, 0.5 N NaOH with 1.5 M NaCl, that denatures double-stranded DNA, so that it can be efficiently transferred to a nylon or nitrocellulose membrane and subsequently hybridized to a labeled probe. When utilized in conjunction with a depurination solution, treatment with the denaturing solution results in the hydrolysis of the phosphodiester backbone at the sites of depurination. This allows for a more efficient transfer of large DNA in neutral Southern transfers.

The Neutralizing Solution, 0.5 M Tris-HCl with 1.5 M NaCl, is used to neutralize the pH of the gel following depurination/denaturation treatment prior to transfer. This allows for a more efficient transfer of DNA in Southern transfers.

Reagents and Equipment Required but Not Provided

- Depurination Solution, 0.2 N HCl
- Neutral Southern Transfer Solution, 0.15 M sodium citrate, tribasic, dihydrate, pH 7.0, with 1.5 M NaCl
- Chromatography paper (Thick paper with medium flow rate and smooth surface) Catalog Number Z270849
- Extra thick blotting paper, Catalog Number P7796
- BioBond membranes
- Molecular Biology Grade water, Catalog Number W4502
- 2× SSC Buffer (0.03 M sodium citrate, pH ~7.0, with 0.3 M NaCl), prepared from Catalog Numbers S6639, S8015, or S0902

Precautions and Disclaimer

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheets for information regarding hazards and safe handling practices.

Storage/Stability

These products may be stored at room temperature for at least 1 year.

Procedure

Neutral Southern Blotting

1. Subject DNA to electrophoresis on an agarose gel (6 × 9 cm) containing the appropriate percentage of agarose to resolve the bands of interest:

large fragments (0.8–10+ kb)	0.7% agarose
medium fragments (0.5–7 kb)	1.0% agarose
small fragments (0.2–3 kb)	1.5% agarose
2. Optional: Stain the gel in a 0.5 µg/ml ethidium bromide solution for 30 minutes at room temperature and visualize with ultraviolet light. BlueView™ (Catalog Numbers T9060 or T8935) may be used for the electrophoresis running buffer, eliminating the need for ethidium bromide staining.

Steps 3-5 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 in several gel volumes of Depurination Solution (0.2 N HCl) for 10 minutes at room temperature.

4. Denaturation – Denature the DNA by soaking the gel for 30 minutes in several gel volumes of Denaturation Solution (Catalog Number N1531).
5. 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 (Catalog Number N1532).
6. While the gel is neutralizing (step 5), prepare membrane and filter paper for transfer. Prepare a blotting wick by cutting a piece of chromatography paper slightly wider and about 5 cm longer than the gel. Wrap the wicking paper around a piece of Plexiglas® 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 (0.15 M sodium citrate, tribasic, dihydrate, pH 7.0, with 1.5 M NaCl) for the entire transfer process. Make sure that both ends of the wick are in good contact with the transfer buffer and that the level of the buffer is below the top of the support. Allow the wick to wet completely and remove any trapped air bubbles. Cut the BioBond membrane and 10 pieces of extra thick blotting paper to the size of the gel. Pre-wet the membrane and one piece of the extra thick blotting paper with Neutral Southern Transfer Solution.
7. Assemble the transfer apparatus for a standard upward capillary transfer. At each step carefully remove air bubbles by rolling a disposable pipette over the surface.
 - a. Place the neutralized gel on the thoroughly wetted wicking paper.
 - b. 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.
 - c. Place the pre-wetted membrane on top of the gel and mark for orientation with a pencil.
 - d. Carefully position the pre-wetted piece of extra thick blotting paper on top of the membrane.
 - e. Top this with the remaining 9 dry sheets of extra thick blotting paper.
 - f. 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 transfers.
 - g. Allow transfer to proceed for 2 hours. When using extra thick 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 extra thick blotting paper; in this case allow the transfer process to proceed for 6-18 hours.
8. 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 2× SSC to remove any agarose that may be stuck to the membrane.
9. Transfer the membrane to fresh pieces of blotting paper 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 ultraviolet light (254 nm).
10. The membranes can be stored at room temperature between clean pieces of blotting paper until needed. The membrane is now ready for probing. Any preferred method of probing may be used.
11. To assess the efficiency of transfer, the gel may be restained in a 0.5 µg/ml ethidium bromide solution for 30 minutes at room temperature and visualized with ultraviolet light.

References

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

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