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Product Information

NEUTRALIZING SOLUTION

For Neutral and Alkaline Southern Transfers

Product No. **N1532**

Product No. **A8092**

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.

This solution neutralizes the effects of depurination and denaturation solutions, allowing for more efficient transfer of DNA in Southern transfers.

Precautions and Disclaimer

Sigma's Neutralizing Solution is for laboratory use only. Not for drug, household or other uses.

Storage/Stability

The product may be stored at room temperature for at least 1 year.

Reagents and Equipment Required but Not Included (Sigma Product Numbers are given where available)

- Depurination Solution for Southern Transfers, Product No. N1907
- Denaturation Solution for Neutral Southern Transfers, Product No. N1531
- Neutral Southern Transfer Solution, Product No. N0907
- Alkaline Southern Transfer Solution, Product No. A7967
- Medium thickness blotting paper, Product No. P6664
- Quickdraw™ extra thick blotting paper, Product No. P7796
- BioBond™ membranes
- Molecular Biology Grade water, Product No. W4502
- 2X SSC buffer (0.03 M sodium citrate, 0.3 M sodium chloride, pH approx. 7.0), prepared from Product No. S6639, S8015 or S0902

Procedure

A. Neutral Southern Blotting

1. Subject DNA to electrophoresis on an agarose gel (6 x 9 cm) 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.
2. Stain gel in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualize with ultraviolet light. BlueView™ (Product No. 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 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.
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.

6. 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 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 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 BioBond membrane and 10 pieces of Quickdraw blotting paper to the size of the gel. Pre-wet the membrane and one piece of Quickdraw 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.
 - 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 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 transfers.

 - 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.
 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 2X 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 254 nm ultraviolet light.
 10. The membranes can be stored at room temperature between clean pieces of blotting paper until needed.
 11. 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.
- B. Alkaline Southern Blotting**
1. Subject DNA to electrophoresis on an agarose gel (6 x 9 cm) 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.
 2. Stain gel in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualize with ultraviolet light. BlueView may be used for the electrophoresis running buffer, eliminating the need for ethidium bromide staining.

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 in several gel volumes of Depurination Solution for 10 minutes at room temperature.
 4. Denaturation: Denature the DNA by soaking the gel for 30 minutes in several gel volumes of Alkaline Southern Transfer Solution.

5. Transfer the DNA by capillary action as described in steps 6 and 7 of the Neutral Southern Blotting standard protocol outlined above substituting Alkaline Southern Transfer Solution for Neutral Southern Transfer Solution in each step.
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 place in Neutralizing Solution for 15 minutes at room temperature to neutralize the membrane and remove any agarose that may be stuck to the membrane.
7. 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 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.

Related Products

PerfectHyb Plus, Product No. H7033
BlueView TBE Buffer, Product No. T9060
BlueView TAE Buffer, Product No. T8935

General References

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

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