

Product Information

QuickDraw Blotting Paper

Product Numbers **P 6928, P 7796, P 8046, P 6803,**
P 8549, P 7921, P 8171, P 7176

QuickDraw[™]
blotting paper 

TECHNICAL BULLETIN

Product Description

QuickDraw Blotting Paper is a convenient alternative to paper towels for capillary Southern, Northern, and Western blots. In a typical Southern blot, DNA will completely transfer in two hours using ten sheets. If an overnight transfer is preferred, five sheets are sufficient for a complete transfer.

QuickDraw Blotting Paper is available in pre-cut sizes to match many commonly used nitrocellulose and nylon membranes.

Procedure

Reagents

- Tris-Borate-EDTA (TBE) Buffer, 5x, Product Number T 6400
0.445 M Tris borate, pH 8.3, 0.01 M EDTA
- Gel Loading Solution, Product Number G 2526
0.05% (w/v) Bromophenol Blue, 40% (w/v) sucrose,
0.1 M EDTA, pH 8.0, and 0.5% (w/v) SDS.
- Denaturation Solution for Neutral Southern Transfer, Product Number N 1531,
0.5 N NaOH and 1.5 M NaCl
- Neutralizing Buffer for Southern blots, Product Number N 6019
Supplied in pouches to prepare 1 L of buffer. After reconstitution with 1 L Water for Molecular Biology (Product Number W 4502), the buffer contains 1 M Tris-HCl, pH 8.0, and 1.5 M NaCl.
- SSC Buffer, 20x, Product Number S 6639
0.3 M sodium citrate, pH 7.0, and 3 M NaCl. Also available in powdered blends.

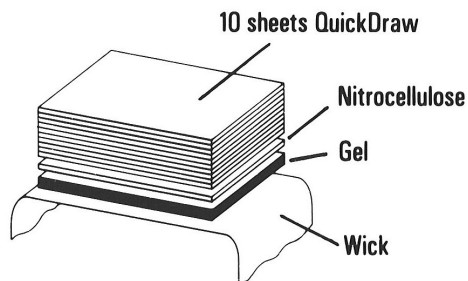
or

- SSPE Buffer, 20x, Product Number S 2015
0.2 M Phosphate buffer, pH 7.4, 2.98 M NaCl,
0.02 M EDTA. Also available in powdered blends.
- Ethidium Bromide, Product Number E 7637,
E 2515, or E4391.
- Medium Blotting Paper, Product Number P 6914 or
P 9039.

Electrophoresis

1. Prepare a 0.7-0.8% agarose gel using 1x TBE.
2. Load the gel with sufficient sample DNA in Gel Loading Solution.
3. Proceed with electrophoresis in 1x TBE buffer until the bromophenol blue is 1-2 cm from the end of the gel.
4. Stain the gel in 0.25 µg/ml ethidium bromide solution for at least 15 minutes. Photograph the gel.
5. Transfer the gel to a glass baking dish or other suitable container.
6. For DNA smaller than 20 kb go to step 7. For especially large DNA (>20 kb), soak the gel twice for 15 minutes in 0.25 M HCl to hydrolyze the DNA. This aids in the transfer of large DNA fragments.
7. Denature the gel by soaking in several volumes of Denaturing Solution for one hour with constant gentle shaking.
8. Neutralize the gel by soaking in several volumes of Neutralizing Solution for one hour with constant gentle shaking.

Blotting



1. Refer to the above diagram for the suggested set-up for effective transfer.
2. Wet the wick (Medium Thick Blotting Paper) in transfer buffer (10x SSC or 10x SSPE) and place over a solid support in a transfer reservoir so the ends will be in the transfer buffer.
3. Fill the reservoir with transfer buffer to just below the top of the support. As buffer is absorbed, add fresh buffer to the reservoir, if necessary, to insure continuous, complete transfer.
4. Invert the gel so the original underside is now uppermost, place on the wick, and smooth to remove all air bubbles between the wick and gel.
5. Cut a sheet of nitrocellulose membrane to exactly the same size as the gel, and wet it by floating in 2x SSC or 2x SSPE for 2-3 minutes.

Note: Handle the nitrocellulose membrane with Teflon[®] coated forceps and wear gloves. Membranes touched by greasy hands will not wet.
6. Cut ten sheets of QuickDraw Extra Thick Blotting Paper to the same size as the nitrocellulose membrane and place on the nitrocellulose. QuickDraw should not extend beyond the edges of the gel. If the QuickDraw is cut larger than the sheet of nitrocellulose, the transfer buffer could be absorbed without first passing through the gel and nitrocellulose.
7. Place a glass plate on top of the QuickDraw and secure the stack with a 500-g. weight. Transfer is

complete after two hours, or when the QuickDraw is completely saturated.

8. If an overnight transfer is preferred, five sheets of QuickDraw Extra Thick Blotting Paper can be used to completely transfer the DNA to the nitrocellulose.
9. Carefully restrain and photograph the gel to verify that all the DNA has been transferred from the gel to the nitrocellulose.
10. The nitrocellulose is now ready for hybridization and detection.

References

Sambrook, J., and Russell, D. W., Molecular Cloning: A Laboratory Manual, 3rd Ed., p. 6.34. (Cold Spring Harbor Laboratory Press, 2001).

Related Products

Thick Blotting Paper

- 20 x 20 cm, Product Number P 6664.
- 33 x 56 cm, Product Number P 9164.

Nitrocellulose Membranes

- 15 x 15 cm, pore size 0.2 μ m, Product Number N 7892
- 20 x 20 cm, pore size 0.2 μ m, Product Number N 8017
- 7 x 10 cm, pore size 0.45 μ m, Product Number N 8142
- 15 x 15 cm, pore size 0.45 μ m, Product Number N 8267
- 20 x 20 cm, pore size 0.45 μ m, Product Number N 8392

SSC Buffer

- Powder blend in foil pouches to prepare 1 L, Product Number S 0902
- Powder blend in bottles to prepare 100 ml or 1 L, Product Number S 8015

SSPE Buffer

- Powder blend in foil pouches to prepare 1 L, Product Number S 1027
- Powder blend in bottles to prepare 100 ml or 1 L, Product Number S 8140

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