

Protocol TurboBlotter™ System

Alkaline Transfer of DNA to Nytran® SuPerCharge Nylon Membranes

Alkaline Transfer Buffers

To make one liter

Denaturing Buffer	Transfer Buffer	Neutralizing Buffer 5x
3 M NaCl, 0.4 M NaOH	3 M NaCl, 8 mM NaOH	1 M phosphate buffer, pH 6.8
175.5 g NaCl	175.5 g NaCl	79.25 g Na ₂ HPO ₄
16.0 g NaOH	0.32 g NaOH	60.25 g NaH ₂ PO ₄ ·H ₂ O
873.0 ml DI H ₂ O	879.0 ml DI H ₂ O	Bring to 1 liter with DI H ₂ O

(This is a 5x concentrate; no pH adjustment is necessary. Make a 1:5 dilution of 5x neutralizing buffer.)

To prevent neutralization by CO₂, store the transfer buffer in a tightly closed glass bottle.

IMPORTANT NOTE: *Alkaline transfers onto Optitran® BA-S nitrocellulose membranes are not recommended with the TurboBlotter system.*

A. Denaturation of DNA gels

1. Incubate the gel in denaturing buffer 2 times, 30 min each. (Shake the dish containing the gel slowly.)
2. Wash the gel in transfer buffer for 15 min.

B. Transfer

1. oak transfer membrane (Nytran® SuPerCharge nylon) in distilled water for 15 min.
2. Place "stack tray" of transfer device on bench, making sure it is level.
3. lace 20 sheets of dry GB004 blotting paper (thick) in stack tray.
4. lace 4 sheets of dry 3MM Chr blotting paper (thin) on top of stack.
5. Place one sheet of prewet 3MM Chr blotting paper in transfer buffer on stack.
6. Place transfer membrane on stack.
7. Cover the membrane with agarose gel; cut the gel to the size of the membrane, making sure there are no air bubbles between the gel and the membrane.

NOTE: *If the gel is smaller than the blotting paper used, cover excess blotting paper with plastic wrap or Parafilm® to prevent wicking of buffer through the paper rather than the gel.*

8. Wet the top surface of the gel with transfer buffer, and place 3 sheets of 3MM Chr blotting paper, presoaked in transfer buffer, on top of the gel.
9. Attach the "buffer tray" of the transfer device to the bottom tray, using the circular alignment buttons to align both trays.
10. Fill the buffer tray with transfer buffer.
 - 125 ml for 7 x 8 cm to 11 x 14 cm transfers
 - 200 ml for 12 x 21 cm to 20 x 25 cm transfers

Protocol TurboBlotter™ System (continued)

11. Start the transfer by connecting the gel stack with the buffer tray using the precut "buffer wick" (included in each blotter stack), presoaked in transfer buffer. Place the wick across the stack so that the short dimension of the wick completely covers the blotting stack and both ends of the long dimension extend into the buffer tray. Place the "wick cover" on top of the stack to prevent evaporation. Make sure the edges of the wick are immersed in the transfer buffer.
12. Continue the transfer for 1 h. Additional transfer time may be required for gels thicker than 4 mm or larger-sized nucleic acids.

NOTE: Do not place any other weight on top of the "wick cover" during transfer. This is unnecessary and may inhibit transfer by crushing the pore structure of the agarose gel.

C. Neutralization

Following transfer, gently wash the transfer membrane in 1x neutralizing buffer (0.2 M sodium phosphate, pH 6.8) for 5 min.

D. Drying (Immobilization)

1. Place the membrane on a fresh sheet of dry 3MM Chr blotting paper to remove any excess of neutralizing buffer.
2. Bake membrane at 80 °C for 20 min–2 hrs.

NOTE: Fixation of DNA requires baking for only 20 min or until the blot has dried completely. As more blots are placed in the oven, more time will be required.

3. As an alternative to drying on Nytran SuPerCharge membrane, the DNA may also be covalently bound to the membrane by cross-linking the molecule to the nylon matrix in the presence of UV light (Church and Gilbert, 1984). Expose the blot to a source of UV light (254 nm) for a total dose of 120 mJ/cm² for a damp membrane.

E. Storage

Store blots desiccated at 4 °C for several months.

F. Hybridization

Hybridize and detect using desired method.

Neutral Transfer of DNA or RNA to Optitran® Nitrocellulose or Nytran® SuPerCharge Nylon Membranes

Neutral Transfer Buffers

To make one liter

Denaturing Buffer

0.5 M NaOH	20 g	NaOH
1.5 M NaCl	87.66 g	NaCl

Bring to 1 L with DI H₂O

Adjust the pH to 7.0; bring to 1L with DI H₂O.

20x SSC Transfer Buffer

3 M NaCl	175.5 g	NaCl
0.3 M Na citrate	88.2 g	Na citrate
(Na ₃ C ₆ H ₅ O ₇ · 2H ₂ O)	800 ml	DI H ₂ O

Neutralizing Buffer

0.5 M Tris-HCl pH 7.0	60.56 g	Tris-HCl pH 7.0
1.5 M NaCl	87.66 g	NaCl
	800 ml	DI H ₂ O

Adjust the pH with concentrated HCl; bring to 1L with DI H₂O.

Protocol TurboBlotter™ System (continued)

A. Denaturation of DNA gels

1. Place gel in denaturing buffer for 30 min at room temperature. (Shake the dish containing the gel slowly.)

NOTE: *If target DNA fragment is >15 kb, then acid depurination may improve transfer. This step precedes the denaturation step. Soak the gel in 0.25 M HCl for 30 min.*

2. Rinse the gel with distilled water, and transfer to neutralizing buffer. Shake gel slowly for 30 min at room temperature.
3. Soak gel in 20x SSC transfer buffer for 30 min. Shake gel slowly.

NOTE: *If DNA fragments are <500 bp, use 20x SSC transfer buffer. If fragments are >500 bp, 10x SSC transfer buffer is a high-enough ionic strength to retain DNA on nitrocellulose.*

B. Treatment of RNA gels

RNA gels that have been run in 2.2 M formaldehyde should be rinsed four times in deionized water and then maintained in low-ionic-strength conditions (DI H₂O or low-salt solution) prior to transfer. Alternatively, if the concentration of formaldehyde is 0.41 M (Chomczynski, 1992), no gel washing is required prior to transfer.

C. Transfer

1. Wet and immerse nitrocellulose or nylon membrane (Optitran BA-S 83 or Nytran SuPerCharge) in distilled water. Then soak membrane in 20x SSC transfer buffer for 5 min.
2. Place "stack tray" of transfer device on bench, making sure it is level.
3. Place 20 sheets of dry GB004 blotting paper (thick) in stack tray.
4. Place 4 sheets of dry 3MM Chr blotting paper (thin) on top of stack.
5. Place one sheet of 3MM Chr blotting paper, prewet in transfer buffer on stack.
6. Place transfer membrane on stack.
7. Cover the membrane with agarose gel; cut the gel to the size of the membrane, making sure there are no air bubbles between the gel and the membrane.

NOTE: *If the gel is smaller than the blotting paper used, cover excess blotting paper with plastic wrap or Parafilm® to prevent wicking of buffer through the paper rather than the gel.*

8. Wet the top surface of the gel with transfer buffer, and place 3 sheets of 3MM Chr blotting paper, presoaked in transfer buffer, on top of the gel.
9. Attach the "buffer tray" of the transfer device to the bottom tray, using the circular alignment buttons to align both trays.
10. Fill the buffer tray with transfer buffer.
125 ml for 7 x 8 cm to 11 x 14 cm transfers
200 ml for 12 x 21 cm to 20 x 25 cm transfers
11. Start the transfer by connecting the gel stack with the buffer tray, using the precut "buffer wick" (included in each blotter stack), presoaked in transfer buffer. Place the wick across the stack so that the short dimension of the wick completely covers the blotting stack and both ends of the long dimension extend into the buffer tray. Place the "wick cover" on top of the stack to prevent evaporation. Make sure the edges of the wick are immersed in the transfer buffer.
12. Continue the transfer for 3 hr. Additional transfer time may be required for gels thicker than 4 mm or larger-size nucleic acids.

NOTE: *Do not place any other weight on top of the "wick cover" during transfer. This is unnecessary and may inhibit transfer by crushing the pore structure of the agarose gel.*

Protocol TurboBlotter™ System (continued)

D. Neutralization

Following transfer, gently wash the transfer membrane in 2x SSC for 5 min.

E. Drying (Immobilization)

1. Place the membrane on a fresh sheet of dry 3MM Chr blotting paper to remove any excess of 2X SSC buffer.
2. Bake membrane at 80 °C for 20 min-2 hrs.

NOTE: *A vacuum oven is recommended for baking of nitrocellulose membranes. Fixation of DNA or RNA requires baking for only 20 min or until the blot has dried completely. As more blots are placed in the oven, more time will be required.*

3. As an alternative to drying on Nytran® SuPerCharge nylon or Optitran® BA-S Nitrocellulose membranes, the DNA or RNA may also be covalently bound to the membrane by cross-linking the molecule to the matrix in the presence of UV light (Church and Gilbert, 1984). Expose the blot to a source of UV light (254 nm) for a total dose of 120 mJ/cm² for a damp membrane.

F. Storage

Store blots desiccated at 4 °C for several months.

G. Hybridization

Hybridize and detect, using desired method.

To make TurboBlotter RNase-free

1. Wash the TurboBlotter device in detergent.
2. Rinse the device completely with DI H₂O.
3. Dry with ethanol (EtOH).
4. Soak TurboBlotter device in 3% hydrogen peroxide (H₂O₂) for 10 min at room temperature.
5. Rinse completely with DI H₂O that has been treated with 0.1% DEPC.

NOTE: *Do not treat the TurboBlotter device directly with DEPC or autoclave. We recommend purchasing two separate devices for doing Northern and Southern transfers.*

Stacking TurboBlotter trays for multiple transfers

For convenience and to save space, the large TurboBlotter systems can be stacked crisscross for multiple transfers.

We recommend that the stacked trays remain stationary on the bench and not be transported from lab to lab while assembled.