NorthernMax® Transfer Buffer

Store at room temperature.

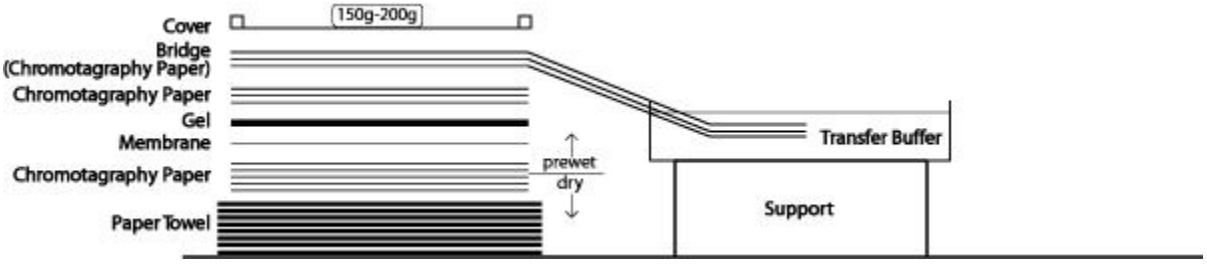
Catalog #:	AM8672
Amount:	1 L
Product Description:	Buffer designed for efficient transfer of RNA to membranes for Northern analysis.
Appearance:	Clear solution
Storage Conditions:	Store at room temperature.

USER INFORMATION

Applications:

Northern Transfer Protocol

This protocol is based on a 0.6 cm thick gel. To assemble a passive downward transfer apparatus, refer to the figure.



We find this procedure to be superior to conventional upward capillary transfer. A commercially available active transfer apparatus (electroblotter, semi-dry gel blotter, vacuum blotter, pressure blotter, etc.) may be used in lieu of the transfer protocol described below. Be sure to follow the manufacturer's recommendations.

1. Remove the unused top portion of the gel by cutting through the wells with a razor blade. This prevents transfer buffer form flowing through the wells instead of the gel during transfer. To ensure proper orientation later, cut a notch into the upper right hand corner.
2. Place 0.5 mL of Transfer Buffer per cm^2 of gel surface into a glass or plastic, flat-bottomed container somewhat larger than the dimension of the agarose gel. This will serve as the reservoir for the transfer buffer and will also be used to wet the blotting paper, membrane, and bridge.
3. Cut a stack of paper towels 1–2 cm wider than the agarose gel and stack tightly 2–3 cm high next to the transfer buffer reservoir.
4. Cut five filter paper sheets (e.g., Whatman 3 MM) the same size or slightly larger than the gel. These are stacked on top of the paper towels. The top-most 2 sheets should be wet in transfer buffer.
5. The membrane should be the same size or slightly larger than the gel. Notch the membrane to match the gel. When cutting the membrane to size, use a razor blade or scalpel. Handle the membrane only by the edges using gloved hands. Wet the membrane briefly in transfer buffer and place it on top of the stack of filter paper sheets. Be sure that there are no bubbles trapped between the layers. A glass rod or Pasteur pipette may be used to gently roll out any bubbles.
6. Lay the gel on top of the wet membrane, again smoothing out any bubbles.

7. Cut 3 sheets of filter paper to the same size as the gel, wet in the transfer buffer, and place on top of the gel. Again, be sure that there are no bubbles trapped between the layers.
8. Cut 3 filter paper bridges large enough to cover the area of the gel and to reach across into the transfer buffer reservoir. Wet these in transfer buffer and place on top of the stack. Make sure there are no bubbles trapped between any of the layers.
9. Cover the stack with the casting tray used to pour the gel. Otherwise, anything flat, rigid, and of similar area will work nicely. Place a 150–200 g object on top to lightly compress the stack. A small beaker filled with 100 mL of water works well.
10. Make sure the filter paper bridge is in contact with the transfer buffer in the reservoir. There should be no path for the transfer buffer to follow from the reservoir to the dry filter paper and paper towels except through the gel. The most common place for "short circuiting" to occur is the area where the bridge enters the stack. If the bridge touches the filter paper or the paper towels underneath the gel, the flow of buffer will bypass the gel and reduce transfer efficiency drastically.
11. Allow the transfer to continue for ten minutes per millimeter of gel thickness. Do not let the transfer proceed for much more than 3 hours as this will begin to reduce hybridization signals.
12. Carefully disassemble the transfer apparatus. Remove the membrane with forceps and briefly rinse in 2X SSC (0.3M NaCl, 0.03M sodium citrate) to remove salt and agarose.
13. The membrane should be treated to crosslink the RNA immediately after transfer and rinsing. This may be done by one of two methods: 1) The membrane may be treated with UV light (Ambion Technical Bulletin #169). If using a commercially available UV crosslinker, follow manufacturer's recommendations. 2) The membrane may be treated by baking at 80°C for 15 minutes.
14. The membrane may be stored wrapped in plastic wrap or in a 50 mL conical vial at 4°C until ready for analysis.

QUALITY CONTROL

Nonspecific Endonuclease Activity:	Meets or exceeds specification when a sample is incubated for 14–16 hr with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.
Exonuclease Activity:	Meets or exceeds specification when a sample is incubated for 14–16 hr with 40 ng ^{32}P-labeled *Sau*3A fragments of pUC19 and analyzed by PAGE.
RNase Activity:	Meets or exceeds specification when a sample is incubated for 14–16 hr with 25 ng ^{32}P-labeled RNA and analyzed by PAGE.
Functional Testing:	NorthernMax® Transfer Buffer is functionally tested using the Ambion® NorthernMax® Kit (Cat #AM1940).

OTHER INFORMATION

Material Safety Data Sheets:

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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