RapidBlot™ Senior Transfer System
RapidBlot™ Junior Transfer System
RapidBlot™ Membrane Packs with BioBond™
or BioBond Plus™ Membranes
Product Codes **RAPID-1, RAPID-2, RP-1, RP-2, RP-3, RP-4**
Technical Bulletin No. MB-1030

ProductInformation

TECHNICAL BULLETIN

RapidBlot™ Transfer Systems	Gel Size	Product Code
RapidBlot Senior Transfer System	11 x 14 cm	**RAPID-1**
RapidBlot Junior Transfer System	7 x 10 cm	**RAPID-2**
RapidBlot™ Membrane Packs with BioBond Plus™ Membranes		
BioBond Plus™ Membrane Packs for RapidBlot™ Senior Transfer System*	11 x 14 cm	**RP-2**
BioBond Plus™ Membrane Packs for RapidBlot™ Junior Transfer System*	7 x 10 cm	**RP-4**
RapidBlot™ Membrane Packs with BioBond™ Membranes		
BioBond™ Membrane Packs for RapidBlot™ Senior Transfer System*	11 x 14 cm	**RP-1**
BioBond™ Membrane Packs for RapidBlot™ Junior Transfer System*	7 x 10 cm	**RP-3**

* RapidBlot™ Membrane Packs include: 5 sheets of BioBond or BioBond Plus membrane, 5 sheets of medium thickness blotting paper, and 80 sheets of QuickDraw™ extra thick blotting paper for the Senior system or 65 sheets for the Junior RapidBlot Transfer system.

Product Description

The RapidBlot™ Senior and RapidBlot™ Junior Transfer Systems are used for downward capillary transfer of nucleic acids from agarose gels to nylon membranes. Traditional Southern blotting transfer involves the upward flow of buffer through the gel with a membrane on top. The buffer is typically drawn up with a large stack of absorbent blotting paper. An advancement in capillary transfer involves downward transfer that reduces transfer time and prevents crushing of the gel. Using the efficient RapidBlot Transfer System, the transfer of nucleic acids from an agarose gel to a nylon membrane can be carried out within one hour versus greater than four hours for the traditional upward capillary method.

The RapidBlot Transfer System can be used in neutral/alkaline Southern blotting and in neutral/mild alkaline Northern blotting. The reservoir tray contains a membrane to govern the flow of transfer solution.

Components Included with the RapidBlot™ Transfer Systems

- RapidBlot Transfer Unit: Base, Stage, and Reservoir Tray 1 set — Product Code R2276 (Senior); Product Code R2401 (Junior)

- BioBond Plus™ Membrane Packs for RapidBlot™ Transfer System, 5 each — Product Code RP-2 (Senior); Product Code RP-4 (Junior)

Precautions and Disclaimer

The RapidBlot Transfer System is for R&D use only. Not for drug, household or other uses.

The RapidBlot is designed for use with 0.7-1.0% agarose gels 3.5-4.5 mm thick. Under these conditions transfer is essentially complete in one hour. Increasing gel thickness and/or agarose concentration may necessitate additional transfer time. This apparatus is not designed for agarose gel concentrations >1.25%.

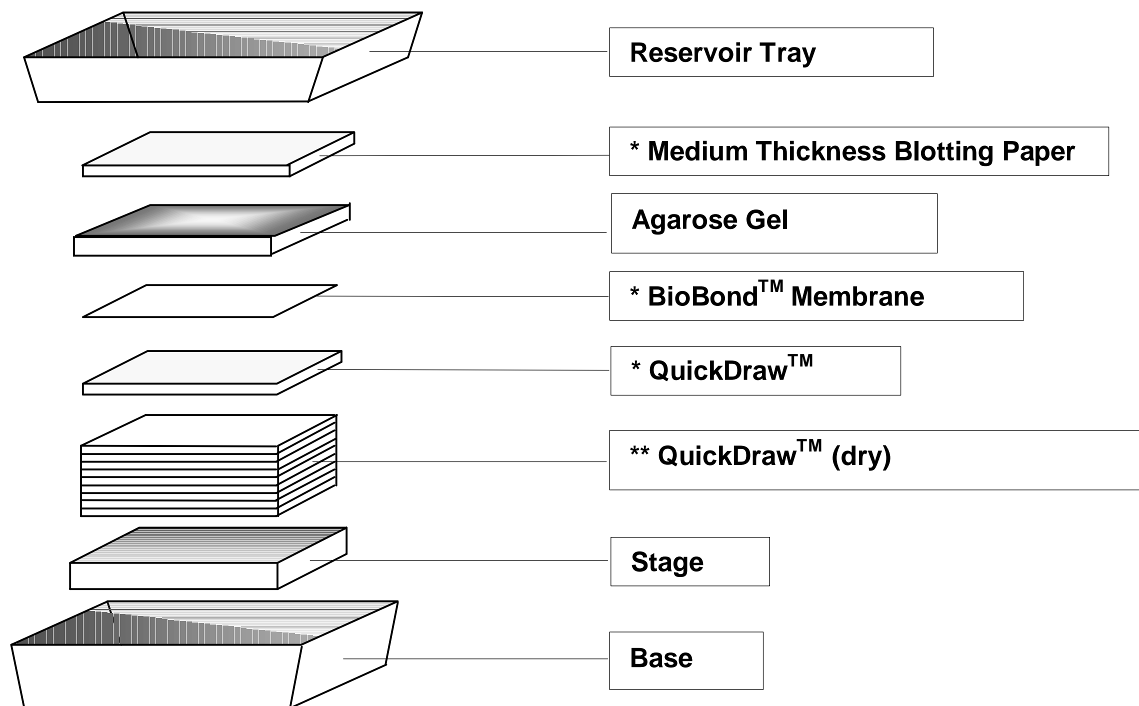
Reagents that May be Required, but are Not Provided

Product Name	Product Code
Depurination Solution for Neutral Southern Transfer	N 1907
Denaturation Solution for Neutral Southern Transfer	N 1531
Neutralizing Solution for Neutral Southern Transfer	N 1532
Transfer Solution for Neutral Southern Transfer	N 0907
Transfer Solution for Alkaline Southern Transfer	A 7967
Neutralizing Solution for Alkaline Southern Transfer	A 8092
Neutral Northern Transfer Solution	N 6531
Mild Alkaline Northern Transfer Solution	A 8217
Mild Alkaline Northern Wash Solution	A 8342
SSC Buffer, 20X Concentrate	S 6639

Storage/Stability
The RapidBlot Transfer unit is made of a high density polyethylene and may be stored at room temperature. The membrane, which governs the flow of the transfer solution, is stable for at least 50 transfers.

Preparation Instructions
1. 2X SSC for DNA solutions. Prepare 2X SSC by diluting 50 ml of 20X SSC with 450 ml of distilled, deionized water.

Figure 1



Assembly Directions
Refer to Figure 1

a. The **Stage** fits at the bottom of the Base.
b. Center 12 sheets for the RapidBlot Senior and 15 sheets for the RapidBlot Junior of dry **QuickDraw**™ blotting paper on the Stage.
c. Pre-wet 1 sheet of **QuickDraw** in the appropriate transfer solution for 10 seconds and place on top of the dry QuickDraw sheets.
d. Pre-wet **BioBond**™ membrane in appropriate transfer solution and place on top of the pre-wet QuickDraw.
e. Center **Agarose gel** on the pre-wet BioBond membrane. Smooth out any air bubbles to allow an even, efficient transfer. Note: Adding extra transfer solution to the membrane before placing the gel reduces the formation of bubbles.
f. Pre-wet **Medium Thickness Blotting Paper** in appropriate transfer solution for 10 seconds, and place on top of the agarose gel.
g. Place **Reservoir Tray** level on top of base and medium thickness blotting paper. Check for contact between the Reservoir Tray and the blotting paper.
h. Fill Reservoir Tray with 170 ml of appropriate **transfer solution** for a RapidBlot Senior or 70 ml for a RapidBlot Junior.

Procedure

A. Neutral Southern Blotting

1. Prepare DNA for analysis by electrophoresis in an agarose gel containing the appropriate percentage of agarose to resolve the bands of interest. For larger fragments (≥1 kb) use 0.7% agarose and for smaller fragments (0.3-3 kb), use 0.8-1.0% agarose. If using BlueView Nucleic Acid Stain, 10X TBE Buffer (Product Code T 9060) or 10X TAE Buffer (Product Code T 8935), see the technical bulletin for those products for details of electrophoresis. Ethidium bromide staining is not necessary for BlueView agarose gels.
2. Stain gel in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualize with ultraviolet light.

   Note: Steps 3-5 should be performed at room temperature with gentle agitation.

3. Depurination: If the fragments of interest are larger than 10 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 or at least until bromophenol blue indicator turns yellow.
4. Denaturation: Denature the DNA by soaking the gel for 30 minutes in several gel volumes of Denaturing Solution.
5. Neutralization: Briefly rinse the gel with deionized water to remove any residual denaturation buffer. Neutralize by soaking the gel for 30 minutes in several gel volumes of Neutralizing Solution.
6. Assemble the RapidBlot Transfer System (refer to Assembly Directions) using **Transfer Solution for Neutral Southern Transfer** (Product Code N 0907).
7. Allow 1 hour for the transfer.
8. After transfer is complete, dismantle the reservoir tray and pour off the extra buffer. Peel off the medium thickness blotting paper and dispose. Carefully remove the gel. Carefully remove the membrane with tweezers and place on blotting paper. Dispose of the QuickDraw sheets. Rinse each tray with deionized water, since crystallization of the transfer solution on the reservoir tray could affect the future performance of the flow-regulating membrane.
9. Rinse the membrane in 2X SSC for several minutes to remove any pieces of gel.
10. Allow membrane 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 130 mJoules of 254 nm ultraviolet light. BioBond Plus nylon membranes do not require crosslinking of the DNA.

11. The membrane can be stored at room temperature between clean pieces of blotting paper until needed. For extended use, store blots desiccated at 2-8°C.

12. To evaluate 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. Prepare DNA for analysis by electrophoresis in an agarose gel containing the appropriate percentage of agarose to resolve the bands of interest. For larger fragments (≥1 kb) use 0.7% agarose and for smaller fragments (0.3-3 kb), use 0.8-1.0% agarose. If using BlueView nucleic acid stain, 10X TBE Buffer (Product Code T 9060) or 10X TAE Buffer (Product Code T 8935), see the technical bulletin for those products for details of electrophoresis. Ethidum bromide staining is not necessary for BlueView agarose gels.

2. Stain gel in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualize with ultraviolet light.

Note: Steps 3 and 4 should be performed at room temperature with gentle agitation.

3. Depurination: If the fragments of interest are larger than 10 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 or at least until bromophenol blue indicator turns yellow.

4. Denaturation: Briefly rinse the gel with deionized water to remove any residual running or depurination buffer. Denature the DNA by soaking the gel for 30 minutes in several gel volumes of Transfer Solution for Alkaline Southern Transfer.

5. Assemble the RapidBlot Transfer System (refer to Assembly Directions) using **Transfer Solution for Alkaline Southern Transfer** (Product Code A7967).

6. Allow 1 hour for the transfer.

7. After transfer is complete, dismantle the reservoir tray and pour off the extra buffer. Peel off the medium thickness blotting paper and dispose. Carefully remove the gel. Carefully remove the membrane with tweezers and place on blotting paper. Dispose of the QuickDraw sheets. Rinse each tray with deionized water, since crystallization of the transfer solution on the Reservoir Tray could affect the future performance of the flow-regulating membrane.

8. Rinse the membrane in 2X SSC for several minutes to remove any pieces of gel.

9. Allow membrane 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 130 mJoules of 254 nm ultraviolet light. BioBond Plus nylon membranes do not require crosslinking of the DNA.

10. The membrane can be stored at room temperature between clean pieces of blotting paper until needed. For extended use, store blots desiccated at 2-8°C.

11. To evaluate 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.

C. Neutral Northern Blotting

Note: Always use standard procedures to prevent RNase contamination throughout this protocol.

To make the RapidBlot unit RNase-free, wash the unit in detergent, rinse completely with deionized water and dry with ethanol. Soak the unit pieces in 3% hydrogen peroxide for 10 minutes at room temperature, then rinse completely with deionized water treated with 0.1% DEPC. **Do not treat RapidBlot unit directly with DEPC or autoclave.** It is recommended to purchase two separate units for doing Northern and Southern transfers.

1. Perform electrophoresis with RNA in formaldehyde or glyoxal/ DMSO gels according to published procedures (e.g. Sambrook, *et al.*). The addition of formaldehyde to MOPS (Product Code M 3183) buffered gels is not required when RNA has been denatured 10 minutes at 65°C in RNA Sample Loading Buffer (Product Code R 4268) prior to loading.

2. For RNA gels containing formaldehyde, wash the gel twice for 15 minutes in several gel volumes of RNase free water to remove residual formaldehyde. Glyoxal/DMSO gels do not require any pretreatment.

3. Stain gel in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualize with ultraviolet light.

4. Assemble the RapidBlot Transfer System (refer to Assembly Directions) using **Neutral Northern Transfer Solution** (Product Code N 6531).

5. Allow 1 hour for the transfer.

6. After transfer is complete, dismantle the reservoir tray and pour off the extra buffer. Peel off and dispose the medium thickness blotting paper. Carefully remove the gel. Carefully remove the membrane with tweezers and place on blotting paper. Dispose of the QuickDraw sheets. Rinse each tray with deionized water, since crystallization of the transfer solution on the reservoir tray could affect the future performance of the flow-regulating membrane.

7. Rinse the membrane in 2X SSC for several minutes to remove any pieces of gel.

8. Allow membrane to air dry several minutes. To permanently affix the RNA to the membrane, bake at 80°C for 30 minutes and/or irradiate the membrane (RNA side toward the light source) with 130 mJoules of 254 nm ultraviolet light. BioBond Plus nylon membranes do not require crosslinking of the RNA.

9. The membrane can be stored at room temperature between clean pieces of blotting paper until needed. For extended use, store blots desiccated at 2-8°C.

10. To evaluate the transfer efficiency, 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.

D. Mild Alkaline Northern Blotting

Note: Always use standard procedures to prevent RNase contamination throughout this protocol. See Section C for detailed instructions on the preparation of the RapidBlot unit.

1. Perform electrophoresis with RNA in formaldehyde or glyoxal/DMSO gels according to published procedures (e.g. Sambrook, *et al.*). The addition of formaldehyde to MOPS (Product Code M 3183) buffered gels is not required when RNA has been denatured 10 minutes at 65°C in RNA Sample Loading Buffer (Product Code R 4268) prior to loading.

2. For RNA gels containing formaldehyde, wash the gel twice for 15 minutes in several gel volumes of RNase free water to remove residual formaldehyde. Glyoxal/DMSO gels do not require any pretreatment.

3. Stain gel in a solution of 0.5 µg/ml ethidium bromide for 30 minutes at room temperature and visualize with ultraviolet light.

4. Assemble the RapidBlot Transfer System (refer to Assembly Directions) using **Mild Alkaline Northern Transfer Solution** (Product Code A8217).

5. Allow 1 hour for the transfer.

6. After transfer is complete, dismantle the reservoir tray and pour off the extra buffer. Peel off the medium thickness blotting paper and dispose. Carefully remove the gel. Carefully remove the membrane with tweezers and place on blotting paper. Dispose of the QuickDraw sheets. Rinse each tray with deionized water, since crystallization of the transfer solution on the reservoir tray could affect the future performance of the flow-regulating membrane.

7. Rinse the membrane in Mild Alkaline Northern Wash Solution for several minutes to remove any pieces of gel.

8. Allow membrane to air dry several minutes. To permanently affix the RNA to the membrane, bake at 80°C for 30 minutes and/or irradiate the membrane (RNA side toward the light source) with 130 mJoules of 254 nm ultraviolet light. BioBond Plus nylon membranes do not require crosslinking of the RNA.

9. The membrane can be stored at room temperature between clean pieces of blotting paper until needed. For extended use, store blots desiccated at 2-8°C.

10. To evaluate the transfer efficiency, 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.

Troubleshooting Guide

Problem	Cause	Solution
Incomplete transfer	DNA fragments are too large	Depurinate gel before transfer or increase the depurination time.
	Gel is too thick (over 4.5 mm)	Use a thinner gel or increase the transfer time.
	Agarose gel concentration is too high	Use a lower concentration or increase the transfer time. This apparatus is not designed for agarose gel concentrations >1.25%.
	Stack is not contacting reservoir tray	Add more height to the bottom of the QuickDraw stack.
	Crushing of the gel	Reduce the height of the QuickDraw stack by removing single sheets of QuickDraw until the stack is the correct height.
	Not enough transfer solution	Add more solution to the reservoir tray.
Uneven transfer	Reservoir tray not level	Make sure the reservoir tray is level during transfer.
High background	Non-specific binding of probe to target nucleic acids	Add sheared, denatured salmon testes DNA (Product Code D 7656) to a final concentration of 100 μg/ml in prehybridization and hybridization solutions
	Wash conditions are not sufficiently stringent	Add an ultra-high stringency wash step (0.1X SSC, 0.1% SDS). Wash for 20 minutes at hybridization temperature. Increase the temperature of the hybridization and/or washes.
Weak or absent signal	Probe was not labeled efficiently	Check that the specific activity of radiolabeled probes is $>5 \times 10^8$ cpm/μg. For non-radioactive probes, check the incorporation of hapten by spotting and detecting serial dilutions of probe in direct comparison to a known standard. If probes are not labeled well enough, remake and confirm adequate incorporation rates.
	Target nucleic acids are not present, have been degraded, or are too low for detection	Run agarose gel electrophoresis to confirm nucleic acids are not degraded. Load more target nucleic acids for blotting. For Northern blots, up to 30 μg of total RNA can be loaded per lane
	Non-radioactive detection system is not working properly	Confirm the enzyme/antibody conjugate is functioning properly by spotting and detecting the labeled probe on nylon membrane. If the enzyme/antibody conjugate is functional, check the chemiluminescent substrate by spotting the enzyme/antibody conjugate on a membrane and detecting with the substrate in question.

General References

Chomczynski, P., One-hour downward alkaline capillary transfer for blotting of DNA and RNA. Anal. Biochem., **201**, 134-139 (1992)
Chomczynski, P. and Mackey, K., One-hour downward capillary blotting of RNA at neutral pH. Anal. Biochem., **221**, 303-305 (1994)
Lichtenstein, A.V., *et al.*, A procedure for DNA and RNA transfer to membrane filters avoiding weight-induced gel flattening. Anal. Biochem., **191**, 187-191 (1990)
Meinkoth, J. and Wahl, G., Hybridization of nucleic acids immobilized on solid supports. Anal. Biochem., **138**, 267 (1984)
Southern, E.M., Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol., **98**, 503-517 (1975)
Sambrook, J, *et al.* Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, New York (2000) (Product Code M 8265)

Related Products

Product Name	Product Code
QuickDraw™ Extra Thick Blotting Paper	P 7796, P 6803, P 7921
Medium Thickness Blotting Paper	P 6664
DNA gel loading solution	G 7654
RNA sample loading buffer	R 4268
10X TBE Buffer	T 4415
MOPS-EDTA-sodium acetate buffer	M 5755
BlueView Nucleic Acid Stain, 10X TBE Buffer	T 9060
BlueView Nucleic Acid Stain, 10X TAE Buffer	T8935
Southern Breeze™ Blotting Kits	SBRZ-1A, SBRZ-1B, SBRZ-2A, SBRZ-2B
Alkaline Southern Breeze™ Blotting Kits	ASBRZ-1A, ASBRZ-1B, ASBRZ-2A, ASBRZ-2B
Northern Breeze™ Blotting Kits	NBRZ-1A, NBRZ-1B, NBRZ-2A, NBRZ-2B
PerfectHyb Plus™ Hybridization Buffer	H 7033
CDP-*Star*™ Universal Detection Kit	U-ALK
All-in-One™ Nick Translation Labeling Mixes	N 8530, N 9155, N 8405, N 9280
All-in-One Random Prime Labeling Mixes	R 7522, R 9647, R 7022, R 9522
BioBond™ Plus Nylon Membranes	N 5281, N 5781, N 5406, N 5531, N 5656
BioBond™ Nylon Membranes	N 1406, N 4031, N 3656, N 3781, N 3906
UniScript™ Transcription Kits	US-T3, US-T7, US-SP6
Precast Agarose Gels	P 5472, P 5722, P 6222, P 5972
Ethidium Bromide, aqueous solution, 500 µg/ml	E 1385

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