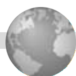

Trans-Blot® SD DNA/RNA Blotting Kit

Instruction Manual

Catalog Number 170-3957

For use with the Trans-Blot SD sem-dry electrophoretic transfer cell (catalog numbers 170-3940, 170-3948, 170-3949)

BIO-RAD

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Note

To insure best results from the Trans-Blot SD DNA/RNA Blotting Kit, become fully acquainted with its instructions before using the kit. It is also recommended that you follow the protocol as closely as possible in order to achieve optimum results.

Bio-Rad recommends that the kit component (gel support frame) be cleaned with a mild, laboratory cleaner (such as Bio-Rad Cleaning Concentrate, catalog number 161-0722) and rinsed thoroughly with distilled water, before use.

Catalog No. ______________________________

Date of Delivery ___________________________

Invoice No. ______________________________

Purchase Order No. _________________________

Warranty

Bio-Rad Laboratories warrants the gel support frame against defects in materials and workmanship for 1 year. If any defects should occur with the frame during the warranty period, Bio-Rad Laboratories will replace the part free of charge. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Damage caused by accident or misuse.
3. Damage caused by disaster.
4. Corrosion due to use of improper solvent or sample.

For an inquiry, contact Bio-Rad Laboratories in the U.S at 1-800-4-BIORAD, outside the U.S. contact your local Bio-Rad office.

Section 1
Introduction

The standard capillary transfer of DNA/RNA from agarose gels to blotting membranes is usually an overnight procedure.[1-3] The Trans-Blot SD DNA/RNA blotting kit, used with the Trans-Blot SD semi-dry transfer cell, allows blotting of DNA and RNA from agarose gels to nylon membrane in only minutes, without any gel pretreatments. The Trans-Blot SD semi-dry electrophoretic transfer cell generates a high field strength (V/cm^2) which facilitates rapid transfers. The blotting kit produces highly efficient transfers by eliminating crushing of agarose gels. PCR* fragments, plasmid, and vector DNA ranging in size from several hundred bases to 15 kilobases can be transferred in as little as 10 minutes. Set-up time is reduced to just minutes because hydrolyzation of DNA fragments is not required prior to transfer. The blotting kit can also transfer RNA to 3.5 kb in 30-35 minutes, although transfer of 28s RNA is equally efficient as determined by EtBr staining.[4]

Read the Trans-Blot SD transfer cell instruction manual thoroughly before performing semi-dry electrophoretic DNA or RNA transfers.

1.1 Specifications

Construction:

Gel support frame — Molded polycarbonate
Overall frame size: — 16 x 21 cm
Maximum gel size: — 15 x 20 cm

Cleaning: Use mild soap and warm water to clean the gel support frame. For cleaning the Trans-Blot SD semi-dry electrophoretic transfer cell, refer to the semi-dry cell instruction manual.

Chemical compatibility: The gel support frame is not compatible with TCA (trichloroacetic acid), chlorinated hydrocarbons (e.g. chloroform), aromatic hydrocarbons (e.g. toluene, benzene), or acetone. Use of such organic solvents voids all warranties.

NOTE: The kit is intended for gels with dimensions of up to 15 x 20 cm. To blot a unique size gel, adjustments must be made in transfer membrane and blot paper dimensions. For questions regarding the proper assembly and operation of the Trans-Blot SD semi-dry electrophoretic transfer cell, refer to the instruction manual provided with the instrument.[6]

Section 2
Preparation for DNA Blotting

1. Prepare enough 0.5x TBE transfer buffer for use in both the horizontal agarose gel electrophoresis and the semi-dry transfer (depending on size of gel). See Appendix for directions.

2. Depending on the size of the DNA fragments to be electrophoresed, pour a corresponding percentage agarose gel with 0.5x TBE (i.e., for genomic DNA use 0.7%, plasmid digests use 0.8% to 1%). We have found that optimal transfer efficiency of plasmid and genomic DNA is achieved using a 0.7% agarose gel, but higher percentage gels can be used with a slight decrease in transfer efficiency. *To insure proper electrode contact, it is important to pour a 6 mm thick gel.* If using a standard gel tray, such as Bio-Rad's UV transparent tray, refer to the chart below to determine the proper volume of agarose needed to pour a 6 mm thick gel. If your gel trays have different dimensions, calculate the volume of agarose required to pour a 6 mm thick gel. Allow the agarose to gel for approximately 30 minutes. Load the gel with your DNA samples and electrophorese in 0.5x TBE buffer.

Gel Dimensions (cm)	Volume Agarose (ml)
7 x 10	42
10 x 15	90
15 x 15	135
15 x 20	180

3. Following electrophoresis, the gel can be stained with ethidium bromide, in 0.5x TBE, and photographed. The gel should remain in 0.5x TBE before transfer to keep the gel properly equilibrated for semi-dry transfer.

Section 3
Preparation for RNA blotting

1. For screening 3.5 kb and smaller RNAs, prepare a 1.2% agarose gel with 1x MOPS and 1.8% formaldehyde. See Appendix for formulas.

2. Aqueous samples of total RNA are heated to 65 °C for 10 minutes, then added to loading buffer. Loading buffer consist of 50% formamide, 6.5% formaldehyde, and 1x MOPS. Typically 5-10 µg of total RNA are loaded per lane. Samples are then heated to 55 °C for 15 minutes; mix 1/10 volume of dye with each sample before loading.

3. Gels are electrophoresed in a submarine apparatus in 1x MOPS buffer until the bromophenol blue dye has migrated ² 10-14 cm. (This usually takes 3-4 hours at 4-5 V/cm[6])

4. Following electrophoresis, the gel is immersed in at least 5 gel volumes of 0.5x TBE buffer containing 0.1 µg/ml EtBr for 30 minutes,[7] changed to 0.5x TBE without EtBr for another 30 minutes. This removes the formaldehyde, stains the gel, and equilibrates the gel for transfer in one step. The gel may be photographed under UV illumination after equilibration. The ribosomal bands can be marked at the edge of the gel by stabbing the gel with an India ink-filled syringe.

   The gel should remain in 0.5x TBE prior to transfer to keep the gel properly equilibrated for semi-dry transfer.

Section 4
General Assembly of Unit for Transfer

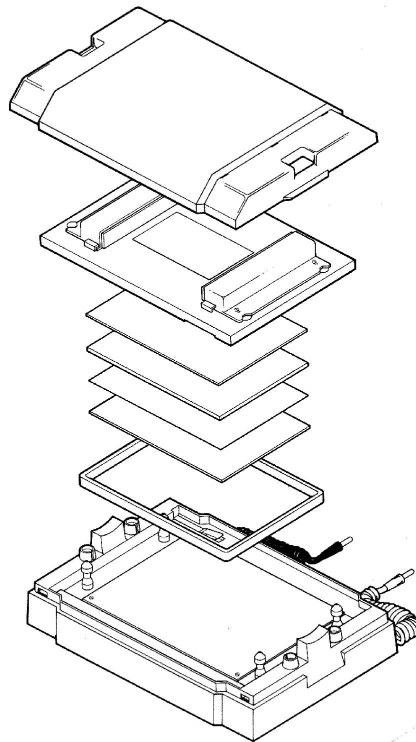
1. Saturate two pieces of precut Extra Thick Blot Paper (provided with the kit) and precut Zeta-Probe® GT membrane (also provided with the kit) or any other transfer membrane in 0.5x TBE buffer. Equilibrate the transfer membrane for at least 10 minutes. The transfer membrane and blot paper must have the same dimensions as the gel for proper transfer to occur. If necessary, cut the transfer membrane and the Extra Thick Blot Paper to the required dimensions with a clean razor blade or scissors. Precut Zeta-Probe GT blotting membrane and extra thick blot paper can be purchased separately.

2. To assemble the transfer sandwich, hold up one piece of blot paper, allow the excess buffer to drain off, and lay it flat on the platinum anode. Using a clean pipette, roll out any air bubbles that may be trapped under the blot paper with a top-to-bottom and left-to-right rolling motion.

3. Place the equilibrated transfer membrane on top of the blot paper and roll out the air bubbles.

4. Carefully place the agarose gel on top of the membrane, well side up. Make sure all the edges are aligned and air bubbles are rolled out.

5. Take the other piece of wetted blot paper, again allowing the excess buffer to drain off, and place it on top of the agarose gel. Roll out the blot paper to remove air bubbles and add approximately 15 ml of transfer buffer (0.5x TBE) on top to resaturate the sandwich. Be sure to remove any excess buffer that is present on the anode electrode surface.



6. Place the support frame around the gel/membrane/blot paper stack and connect the cathode electrode by locking it into place without disturbing the stack. The frame accomodates a 6 mm thick gel, transfer membrane, and two pieces of the Extra Thick Blot Paper provided with the kit and available from Bio-Rad.

7. Place the safety cover onto the unit and plug the Trans-Blot SD cell into the power supply. Be sure to maintain normal polarity of the electrodes, i.e. red lead to red outlet and black lead to black outlet.

   Caution: Do not reverse the electrode polarity. This will damage the stainless steel cathode.

8. Turn on the power supply. The power conditions and transfer times will vary depending on what type and size of DNA/RNA you transfer.

   For plasmid or vector DNA and PCR fragments, set the transfer conditions for a constant 3.55 mA/cm2 of gel area for 10 minutes (i.e. a 7.5 x 15 cm gel with an area of 112.5 cm^2 will require 400 mA constant current for 10 minutes). Normally, the voltage will slowly increase during transfer in order to maintain constant current (i.e., a 7.5 x 15 cm gel running at a constant 3.55 mA/cm^2 will experience a voltage increase of approximately 5 V to 10 V during the transfer).

   For transferring RNA, set the power supply for 3 mA/cm^2 constant current. Typical transfer time should be 30-35 minutes. During the course of the run, the voltage should be about 20 V.

   During the transfer, observe the voltage for any significant fluctuations. The voltage will slowly increase during transfer to maintain constant current. If the voltage is lower, increase the length of the transfer time. If the voltage increases significantly (i.e. greater than 25 V) the buffer capacity has expired and the run should be stopped. If the run is not stopped the gel will overheat and eventually melt. If the voltage requirement is significantly lower than normal, the buffer may be more concentrated than 0.5x, and, therefore, less voltage is required to maintain the specified current. If this is the case, the recommended transfer can be completed as long as the power supply is adjusted to operate at the specified current setting. *Care must be taken not to overheat the gel or the Trans-Blot SD cell.*

9. Following transfer, turn off the power supply and disconnect the leads. Remove the safety cover and the cathode electrode. Discard the blot paper and recover the transfer membrane. Rinse the membrane in 2x SSC. The transfer efficiency can be qualitatively monitored by restaining the gel and checking for any remaining DNA/RNA.

10. To fix the DNA, saturate a piece of blot paper with 0.4 N NaOH. Place the transfer membrane on top of the saturated pad (DNA side up) for 5 minutes. Rinse the membrane briefly in 2x SSC, and bake for 30 minutes at 80 °C in a vacuum oven.

    To fix RNA, dry the membrane it in a 60 °C oven for 2 hours, or dry it overnight at room temperature. The membrane is now ready for hybridization. *Refer to the hybridization procedure in the Zeta-Probe GT blotting membrane instruction manual.*

Section 5 Troubleshooting Guide

A. Poor Electrophoretic Transfer

1. DNA/RNA molecules remain in the agarose gel.

   a. If the gel is hot, the buffer may be too concentrated. The gel is carrying too much current and has begun melting. Buffer concentration must be 0.5x TBE to maintain proper transfer conditions. Remake the transfer buffer.

b. Power conditions during transfer may have changed. It is important to have constant current during the course of the run. If the buffer is less concentrated than 0.5x, higher voltage will be required to maintain the recommended current. If the voltage limit is not set high enough, the current will drop below the optimum range during the run, thereby reducing DNA/RNA migration. Readjust the power supply parameters.

c. Optimum transfers of plasmid, vector, and PCR DNA are achieved when blotting the gel at a constant current of 3.55 mA/cm^2 for the recommended time of 10 minutes. Optimum transfers of RNA are achieved when blotting the gel at a constant current of 3 mA/cm^2 for 30-35 minutes. More difficult transfers (i.e. genomic DNA) may require slightly longer transfer conditions at lower current.

2. Poorly blotted or diffused transfer.

a. There may be a poor contact between the agarose gel and the transfer membrane. Roll out the gel with a pipette prior to transfer to get rid of air or buffer bubbles. Also be sure to remove air and buffer bubbles from the blot paper.

b. The gel may be too thin, causing uneven electrical contact between the gel stack and the electrodes. The gel must be 6 mm thick, and Bio-Rad's ExtraThick Blot Paper must be used with the kit to insure proper electrical contact.

c. The gel may be too hot and is partially melting. See Section A 1-a of troubleshooting.

d. Very small fragments of DNA tend to diffuse during electrophoresis and blotting even if run in high percent agarose gels. Resolution is also not necessarily improved with high percentage agarose gels.

e. The transfer membrane being used may not properly bind DNA or RNA. Try using a control membrane, or a different lot, or a different brand of membrane.

B. Poor Detection Sensitivity

1. Poor DNA probe labeling.

a. Not enough signal is hybridized to the target DNA for detection. Labeled DNA probe may not be properly labeled. Check labeling reaction controls to be sure correct template DNA is being used and that reaction is working properly.

b. Target DNA may not have completely transferred to the membrane. See Section A of troubleshooting. Check agarose gel for presence of DNA following transfer to determine whether transfer occurred or not.

c. Specific activity of the probe may not be high enough for standard detection conditions. Determine specific activity and total cpm of probe added during hybridization.

d. Hybridization conditions may be too stringent. Alter the hybridization conditions to reduce stringency. For more specific recommendations, refer to the Zeta-Probe GT instruction manual.

C. High Background

1. Increase stringency of hybridization conditions to reduce non-specific probe binding.

Section 6
Appendix

10x TBE (per liter*), pH 8.3

Quantity	Final 0.5x Concentration
108 g Tris base	44.5 mM Tris base
55 g boric acid	44.5 mM boric acid
40 ml 0.5 M EDTA, pH 8.0	1 mM EDTA

To make a 0.5x TBE working buffer, mix 50 ml of the 10x TBE stock per 1 liter of deionized, distilled water.

* One liter of Bio-Rad premixed 10x TBE is included with the kit.

1x MOPS, 1 L

Quantity	Final 1x Concentration
4.186 g MOPS	20 mM 3- (N- Morpholino) propanesulfonic acid, pH 7.0
0.41 g sodium acetate	5 mM sodium acetate
2 µl of 0.5 M EDTA, pH 8.0	1 mM EDTA

Dissolve 4.186 g MOPS, 0.41 g sodium acetate, and 2 µl EDTA in 800 ml of ddH_20; adjust volume to l liter with ddH_20.

37% formaldehyde stock solution

1.8% formaldehyde - 5 ml of 37% stock/ 100 ml
6.5% formaldehyde - 1.75 µl of 37% stock/ 10 µl

20x SSC, 1 L

Quantity	Final 20x Concentration
175.3 g NaCl	3 M NaCl
88.2 g sodium citrate	0.3 M sodium citrate

Adjust pH to 7.0 and bring volume to 1 liter with deionized, distilled water. Dilute the 20x stock solution accordingly to make a 2x working solution.

Gel loading buffer - Bromophenol blue dye

1mM EDTA, pH 8.8

0.25% bromophenol blue

0.25% xylene cyanol

50% glycerol

Section 7
Equipment and Accessories

Kit Components

161-0733	Tris/Boric Acid/EDTA, 1 L
170-3960	Extra Thick Blot Paper, 15 x 20 cm, 30
	Gel Support Frame
	Trans-Blot SD DNA/RNA Blotting Protocol

DNA/RNA Blotting Kit Accessories

170-3958	Extra Thick Blot Paper, 10 x 15 cm, 30
170-3959	Extra Thick Blot Paper, 15 x 15 cm, 30
170-3960	Extra Thick Blot Paper, 15 x 20 cm, 30

Trans-Blot SD Electrophoretic Transfer Cells

170-3940	Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell, complete unit
170-3948	Trans-Blot SD System, 100/120 V, includes Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell and Model 200/2.0 power supply, 100/120 V
170-3949	Trans-Blot SD System, 220/240 V, includes Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell and Model 200/2.0 power supply, 220/240 V

Premixed Buffer

161-0733	10X Tris/Boric acid/ EDTA, 1 L
161-0756	10X Tris/Boric acid/EDTA, 6 x 1 L

Power Supplies

165-4761	Model 200/2.0 Constant Voltage Power Supply, 100/120 V
165-4762	Model 200/2.0 Constant Voltage Power Supply, 220/240 V

Zeta-Probe GT Nylon Blotting Membrane

162-0191	Sheets, 10 x 15 cm, 15
162-0192	Sheets, 15 x 15 cm, 15
162-0193	Sheets, 15 x 20 cm, 15

Section 8
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* PCR is covered by U.S. patent number 4,683,202, issued to Cetus Corporation.

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