Southern Blot Procedure for iBlot™ Dry Blotting System

QUICK REFERENCE CARD

Catalog no. IB8010-01

25-1039 Version A; 6 August 2008

The iBlot™ and iBlot™ DNA Transfer Stacks are used for Southern transfer of DNA from gels. The stacks consist of an anode stack with a positively charged 0.2 µm nylon membrane, a cathode stack, and a disposable sponge. Membranes are compatible with subsequent alkaline phosphatase based chemiluminescence or chromogenic detection, as well as with radioactive detection protocols. Instructions for using the iBlot™ Gel Transfer Device to perform Southern blotting of DNA is described below. For detailed instructions, refer to the manual supplied with the iBlot™ Gel Transfer Device or download the manual from www.invitrogen.com.

Gel Electrophoresis

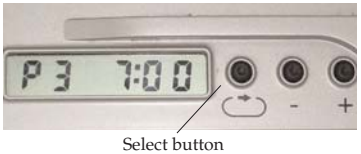
Prepare DNA sample and separate fragments by size using gel electrophoresis according to your standard protocol. For best results, the agarose gel should not be thicker than 7–8 mm.

After electrophoresis, you may need to cut the gel in order to fit it to the size of the iBlot™ Gel Transfer Stack. The maximum size of the gel should not exceed 135 x 77 mm (the blotting surface of the iBlot™ Gel Transfer Device). Trim the gel to the correct dimensions by cutting off the wells and edge sections that do not contain your DNA of interest.

Important: Do not denature or depurinate the gel before transfer.

Selecting a Program

1. Press the power switch to turn **ON** the iBlot™ Gel Transfer Device. The fan in the device begins to run and the digital display shows default parameters (P 3.0 7:00) or the last program used.
2. Press the **Select** button to select the P8 program. Use the Up/Down (+/-) Buttons for changing the values to the recommended parameters listed under **Performing Blotting**.



Select button

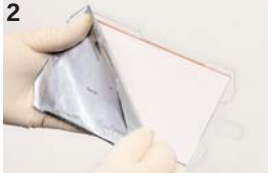
Downloading Upgrades

For users of iBlot™ with older firmware versions that do not have the P8 program, download new iBlot™ firmware versions for running the P8 program at www.invitrogen.com/iblot. Follow instructions on the page to upgrade your device.

Using the iBlot™ Gel Transfer Device for Southern Blotting

Instructions are provided below to assemble the iBlot™ Gel Transfer Device for Southern blotting of DNA gels. **Do not** use the De-bubbling Roller for this protocol.

1. Power on the device using the **on/off** switch at the rear of the unit. Open the lid of the device.



2. Remove the sealing of the iBlot™anode stack (Bottom). **Keep the stack in the plastic tray**.

3. Place the Anode Stack **with the tray** directly on the blotting surface. Align with the gel barriers on the right.

4. Place the gel on the transfer membrane (of the anode stack) with wells facing up and align with upper edge of anode stack.

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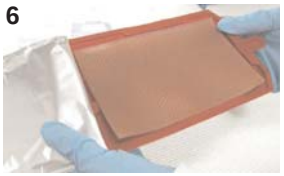
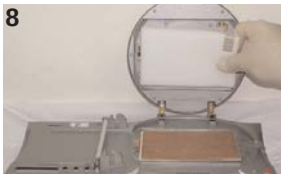
5. Remove any air bubbles with the Blotting Roller.



6. Remove the sealing of the Cathode Stack (Top). Discard the red plastic tray.

7. Place the Cathode Stack over the gel with the electrode side facing up and aligned to the right edge. Remove air bubbles using the Blotting Roller.



8. Position the Disposable Sponge so the metal contact is at the upper right corner of the lid. Proceed to **Performing Blotting**, below.

Performing Blotting

Perform blotting within 15 minutes of assembling the stacks with the gel.

For best results, transfer time should be optimized for your sample. Start with 7 minutes for agarose and acrylamide gels. Use 2–5 minutes for transfer of E-Gels.

1. Close the lid and secure the latch. The red light is on indicating a closed circuit.

2. Select the P8 program and set the time to 7 minute transfer.

3. Press the **Start/Stop** button. The red light changes to green.

4. Current automatically shuts off at the end of each run. The end of transfer is indicated by beeping sounds, and flashing red light and digital display. Press and hold the Start/Stop Button. The light turns to a steady red and the beeping stops.

Disassembling the iBlot™ Gel Transfer Device

Disassemble the device immediately after the end of the blotting procedure.

1. Open the lid of the iBlot™ Device.
2. Discard the iBlot™ Disposable Sponge and iBlot™ Cathode Stack, Top.
3. Carefully remove and discard the gel. Remove the transfer membrane from the stack and proceed with the denaturation step.
4. Discard the iBlot™ Anode stack, Bottom.

Denaturation Step

1. Prepare denaturing solution consisting of 0.4 N NaOH or 1.5 M NaCl/0.5 N NaOH. Make enough to immerse your membrane.
2. Incubate membrane in denaturing solution on a rotary shaker for 10 minutes immediately after transfer. Denaturation of the DNA occurs on the membrane.
3. Air dry the membrane for 5-10 minutes.
4. UV crosslink membrane. Store crosslinked membrane or proceed to hybridization protocol.

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