QUICK REFERENCE

iBlot® Dry Blotting System

Online Specials

Cat. no. IB1001, IB1001EU, IB1001UK

Rev. Date: 2 December 2011

Part no. 25-0912 MAN0003281

Instructions for using the iBlot® Gel Transfer Device to perform dry blotting of proteins from mini- or midi-gels with iBlot® Gel Transfer Stacks is described below. For detailed instructions, including instructions for transfer of proteins from E-PAGE™ gels, transfer of DNA from agarose gels or polyacrylamide gels to iBlot® DNA Transfer Stacks, and performing western detection with iBlot® Western Detection Stack, refer to the manual supplied with the product or download the manual from www.lifetechnologies.com.

General Guidelines

- Remove air bubbles as indicated in the protocol using the Blotting Roller.
- Do not trim the membrane or iBlot® Gel Transfer Stacks to fit your gel size. Use the iBlot® Gel Transfer Stacks, Regular for blotting E-PAGE™, 1 midi-, or 2 mini-gels. Use iBlot® Gel Transfer Stacks, Mini for blotting 1 mini-gel.
- Pretreatment of the gel after electrophoresis is generally not required, but transfer is improved for proteins >150 kDa by equilibriation of the gel in 20% ethanol for 5–10 minutes prior to the transfer.
- To transfer a protein of interest that is >150 kDa, it may be necessary to use Program P3 with a Run Time of 8–10 minutes.
- To transfer a protein of interest that is <30 kDa, it may be necessary to use Program P3 with a Run Time of 5–6 minutes.

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 digital display shows default parameters (P 0 7:00) or the last program used.
2. Press the Select button to select the appropriate program. Use the Up/Down (+/-) Buttons for changing the values to the recommended parameters listed above.

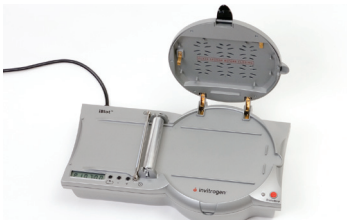
The iBlot® Gel Transfer Device is pre-programmed with 10 voltage programs listed below:

Program	Voltage	Default Run Time	Run Time Limit	Program	Voltage	Default Run Time	Run Time Limit
P0	20 V for 1 min 23 V for 4 min 25 V for remainder	7 min	13 min	P6	7.5 V	3 min	25 min
				P7	5 V	3 min	25 min
P1	25 V	6 min	10 min	P8	20 V for 2 min 23 V for 2 min 25 V for remainder	7 min	13 min
P2	23 V	6 min	11 min				
P3	20 V	7 min	13 min				
P4	15 V	7 min	16 min	P9	20 V for 2 min 5 V for 3 min (x2)	8 min	8 min
P5	10 V	7 min	25 min				

The recommended settings for running a Mini Transfer Stack with a Novex® mini-gel (1.0 or 1.5 mm thick), or a Regular Transfer Stack with an E-PAGE™ *48* Gel, E-PAGE™ *96* Gel, Novex® midi-gel (1 mm thick), or 2 Novex® mini-gels (1.0 or 1.5 mm thick) is **P0 for 7–8 minutes** for **nitrocellulose** stacks, and **P3 for 7–8 minutes** for **PVDF** stacks. Select P8 for running iBlot® DNA Transfer Stacks, and P9 for running iBlot® Western Detection Stacks.

Using the iBlot® Gel Transfer Device with Blotting Roller

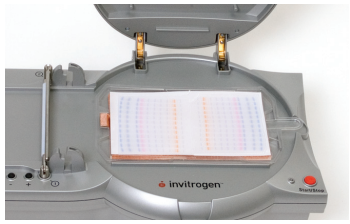
Instructions are provided below to assemble the iBlot® Gel Transfer Stack to perform blotting of mini-, midi-, or other gels using the iBlot® Gel Transfer Device. Refer to the manual if you are using the iBlot® Gel Transfer Device with the de-bubbling roller.



1. Open the lid of the device.

2. Unseal the Anode Stack, Bottom. **Keep the stack in the plastic tray**.

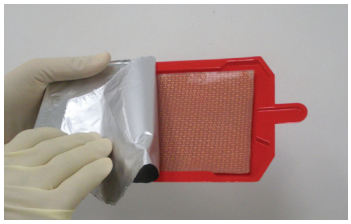
3. Place the Anode Stack (bottom; in the tray) on the blotting surface. Align it with the Gel Barriers on the right.



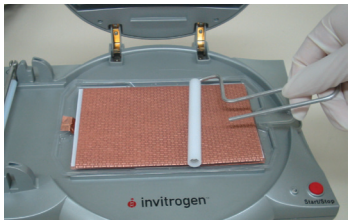
4. Place the pre-run gel(s) on the transfer membrane of the Anode Stack (bottom).

Using the iBlot® Gel Transfer Device with Blotting Roller, continued

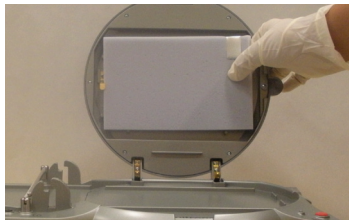
5. Place the pre-soaked (in deionized water) iBlot® Filter Paper on the pre-run gel and remove air bubbles using the Blotting Roller.



6. Unseal the Cathode Stack, Top (shown here for a Mini Stack). Discard the red plastic tray.



7. Place the Cathode Stack, Top over the pre-soaked Filter paper with the electrode side facing up and aligned to the right edge. Remove air bubbles using the Blotting Roller.



8. Place the Disposable Sponge with the metal contact on 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.

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

2. Ensure the correct program and time are selected.

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 release the Start/Stop Button. The light turns to a steady red.

Disassembling the iBlot® Gel Transfer Device

1. Open the lid of the iBlot® Device.
2. Remove the iBlot® E-PAGE™ Tab (used for blotting E-PAGE™ gels only). Rinse the tab with deionized water and store in a dry place for future use.
3. Discard the iBlot® Disposable Sponge and iBlot® Cathode Stack, Top.
4. Carefully remove and discard the gel and filter paper. Remove the transfer membrane from the stack and proceed with the blocking procedure or stain the membrane.
5. Discard the iBlot® Anode stack, Bottom.
6. At this point, the iBlot® Gel Transfer Device is ready for another run (no cooling period is required). If you are not using the device, turn off the power switch.

Downloading Upgrades

To download iBlot® Device firmware upgrades, go to www.lifetechnologies.com/iblot. Follow instructions on the page to download the upgrades.

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