iBlot™ 2 Dry Blotting System

Catalog Number IB21001
Publication Number MAN0009113 **Rev.** C.0

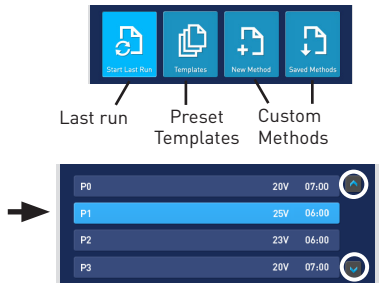
Instructions for using the iBlot™ 2 Gel Transfer Device to perform dry blotting of proteins from mini or midi gels with iBlot™ 2 Transfer Stacks are described below. For detailed instructions refer to the manual at thermofisher.com/iblot2

General guidelines

- **Read "Unpacking Instructions" in the manual for set up instructions when using the iBlot™ 2 Gel Transfer Device for the first time.**
- Use the Blotting Roller to remove any bubbles between layers of the stack.
- Do not trim the membrane or iBlot™ 2 Transfer Stacks to fit your gel size.
- Use the iBlot™ 2 Transfer Stacks, Regular for transferring E-PAGE™, 1 midi (1 mm thick), or 2 mini gels (1.0 or 1.5 mm thick).
- Use iBlot™ 2 Transfer Stacks, Mini for transferring 1 mini gel (1.0 or 1.5 mm thick).
- Method **P0 for 7 minutes** is recommended for transferring most proteins (30–150 kDa) with **nitrocellulose** and **PVDF** stacks.
- Based on the initial results, you can increase or decrease the transfer time to optimize results.
  - A Run Time of 5–6 minutes may be necesssary for transferring proteins of interest <30 kDa.
  - A Run Time of 8–10 minutes may be necesssary for transferring proteins of interest >150 kDa.
- (Optional) Equilibration of the gel in 20% ethanol for 5–10 minutes prior to transfer may increase overall protein transfer efficiency.

Select a method

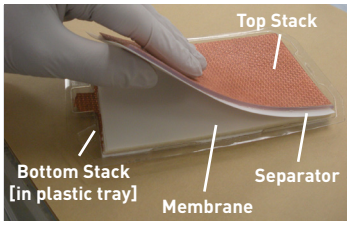
1. Press the power switch to turn ON the iBlot™ 2 Gel Transfer Device. The internal fan should begin running, and the digital display should show the available action icons for selection.
2. Use the up/down arrows to scroll through the list of available methods. Touch the appropriate icon to select a method.



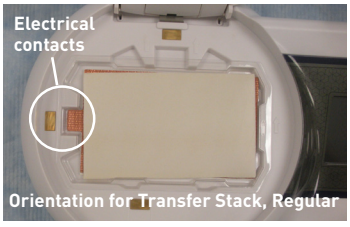
The iBlot™ 2 Gel Transfer Device is pre-programmed with the six preset templates listed below:

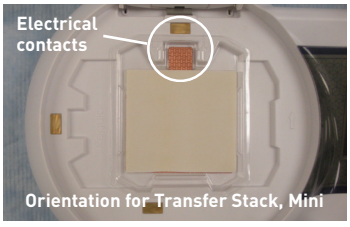
Method	Voltage	Default Run Time	Recommmended Run Time Limit
P0	20 V for 1 min 23 V for 4 min 25 V for remainder	7 min	13 min
P1	25 V	6 min	10 min
P2	23 V	6 min	11 min
P3	20 V	7 min	13 min
P4	15 V	7 min	16 min
P5	10 V	7 min	25 min

Assemble iBlot™ 2 Transfer Stacks on the iBlot™ 2 Gel Transfer Device

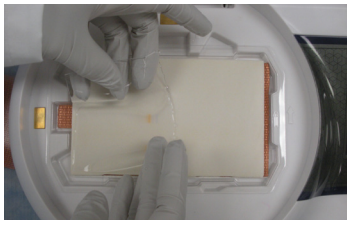


1. Unseal the Transfer Stack and separate into top and bottom halves. Set the Top Stack to one side. Make sure the membrane is not stuck to the separator.
   Keep the Bottom Stack in the plastic tray.





2. Place the Bottom Stack (**in the plastic tray**) on the blotting surface. Align electrical contacts on the tray with the corresponding electrical contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device. Ensure the tray is centered so that the electrical contacts are not obstructed.



3. Wet the pre-run gel(s) and place it on the transfer membrane of the Bottom Stack.

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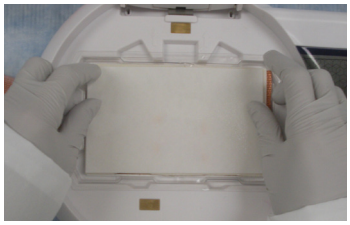
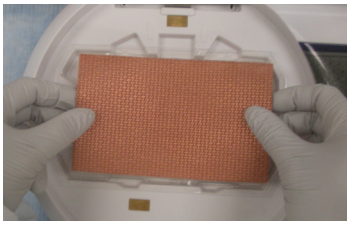
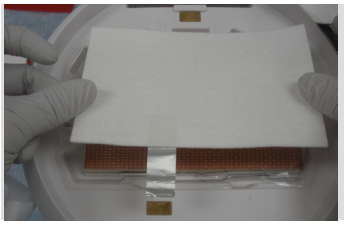
Assemble iBlot™ 2 Transfer Stacks on the iBlot™ 2 Gel Transfer Device, continued



4. Place a pre-soaked (in deionized water) iBlot™ Filter Paper on the gel and remove air bubbles using the Blotting Roller.



5. Remove the white separator and place the Top Stack over the pre-soaked filter paper.
6. Remove air bubbles using the Blotting Roller.

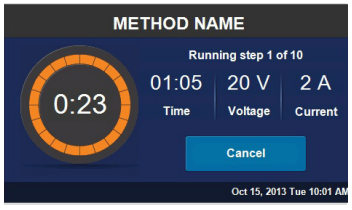


7. Place the Absorbent Pad on top of the Top Stack such that the electrical contacts are aligned with the corresponding electrical contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device.
8. Close and latch the lid of the device carefully to ensure that the Transfer Stack and electrical contacts do not shift.
9. Proceed to "Perform transfer", below.

Perform transfer

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

1. Select the desired method and make sure the parameters are correct.
   Select **Start Run**, or if desired, use the **Start Last Run** icon.



2. Once transfer begins, the elapsed time is displayed on the screen.

3. An audible alarm and a message on the digital display indicates the end of the transfer.
   Select **Done** to end the run.

Disassemble the iBlot™ 2 Transfer Stack

1. Turn off the iBlot™ 2 Gel Transfer Device.
2. Open the lid of the iBlot™ 2 Gel Transfer Device.
3. Discard the Absorbent Pad and Top Stack.
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 Bottom Stack.
6. Clean the instrument surfaces and electrodes with a damp cloth or paper tissue.
7. At this point, the iBlot™ 2 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.

Download upgrades

To download iBlot™ 2 Gel Transfer Device firmware upgrades, go to thermofisher.com/iblot2. Follow instructions on the page to download the upgrades.

For support visit **thermofisher.com/techresources** or email **techsupport@lifetech.com**

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13 October 2016

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