iBlot™ 3 Western Blot Transfer System

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This quick reference card is intended as a benchtop reference for experienced users to perform dry protein blotting from mini and midi gels using iBlot™ 3 Transfer Stacks in the iBlot™ 3 Western Blot Transfer Device. For detailed instructions, refer to the user guide at **thermofisher.com/iBlot3**.

Assemble the iBlot™ 3 Transfer Stack

Each iBlot™ 3 Transfer Stacks box contains 10 stacks, 10 filter papers, and 10 absorbent pads. Assemble the stack according to the steps shown below. Use the blotting roller to remove air bubbles between layers of the stack. Perform the transfer within 5 minutes of assembling the stack with the gel.

Note: If using iBlot™ 3 Transfer Stacks, Low Fluorescence PVDF, you must activate the membrane before use. For more information, see "General Guidelines" table on the reverse side of this document.

1. Unseal the stack, then place the divider with the top stack (cathode) to the side.

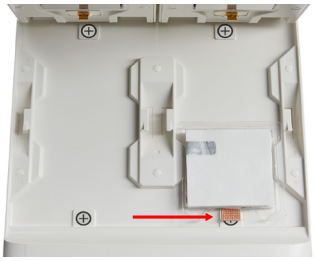
If using iBlot™ 3 Transfer Stacks, Low Fluorescence PVDF, place the activated membrane on top of the bottom stack (anode).[1]

2. Place the separation gel on the transfer membrane of the bottom stack (anode).[1]

3. Briefly wet one filter paper in deionized water and place on top of the gel.[1]

4. Place the top stack without the divider on top of the filter paper.[1]

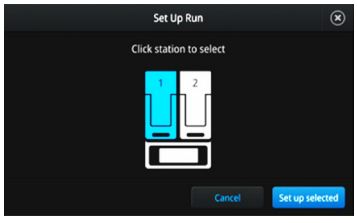
5. Place the absorbent pad on top of the stack such that the side with the longer aluminum tab is facing up and is opposite the copper tab, as shown.



6. Place the stack including the tray on the instrument blotting surface. Align the copper tab with the ⊕ symbol.[2]

7. Close the instrument lid.

8. Press **Set up run** on either station to begin.



9. Select Station 1, Station 2, or both, then press **Set up selected**.

10. Select an existing method or press **Create new** to set up a custom method.

11. Press **Start run**.

12. Transfer is complete when message displays and alarm sounds.

Open lid and disassemble the stack.

Remove the membrane.

[1] Use blotting roller to remove air bubbles between stack layers.

[2] Both midi and mini sized stacks can be aligned to any of the four ⊕ symbols.

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General Guidelines	
Transfer Stacks	iBlot™ 3 Transfer Stacks are not compatible with the iBlot™ 2 Gel Transfer Device.
	iBlot™ 3 Transfer Stacks are supplied pre-assembled in a plastic transfer tray. The plastic tray is placed into the device during the transfer. Discard the plastic tray after the transfer has been completed.
	iBlot™ 3 Transfer Stack bottom (anode) and top (cathode) are separated by a white plastic divider. Discard the divider after assembling the transfer stack.
	For best results, be sure to evenly roll the entire transfer stack using the blotting roller between steps 2–5 and ensure that the transfer stack is even and level before initiating a protein transfer run.
	If using the iBlot™ 3 Transfer Stacks, Low Fluorescence PVDF, note that the membrane is not part of the pre-assembled stack, but is supplied in a pouch that is included in the box. Before use, activate the membrane by incubating it in 100% methanol or 100% ethanol for 3 minutes. Rinse the activated membrane in deionized water before proceeding to iBlot™ 3 transfer stack assembly.
Gel and Membrane	Do not trim the membrane or Transfer Stack to fit your gel size.
	If using the iBlot™ 3 Transfer Stacks, Low Fluorescence PVDF, note that the membrane is hydrophobic and buoyant. After activation in 100% methanol or 100% ethanol, rinse the activated membrane in deionized water, and ensure that it sinks and properly interacts with the water or aqueous buffer.
Washing	For Novex™ Tris-Glycine gels, wash for 5 minutes in deionized water (to remove residual SDS and buffer salts) prior to transfer.
	For targets >150 kDa, wash gel for 5 minutes in deionized water prior to transfer for all gel types.
Cooling	- No cooling is recommended for transfer of high molecular weight proteins. - Low cooling is recommended for broad range molecular weight proteins. - Medium cooling is recommended for low molecular weight proteins.

Pre-Programmed Method	Transfer Parameters
Broad Range (30–250 kDa)	25 V, 6 minutes, Low Cooling
High Molecular Weight (150–400 kDa)	30 V, 8 minutes, No Cooling
Low Molecular Weight (10–120 kDa)	25 V, 3 minutes, Medium Cooling

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

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