

Troubleshooting- eBlot® Protein Transfer System

Problem	Cause	Solution
The right Status Light doesn't flash during blotting process.	Incomplete electric circuit due to improper assembly of the transfer pads.	Ensure the transfer stack is assembled correctly: use the eBlot® Anode Pad first followed by the membrane, the pre-run gel, Gel Window and eBlot® Cathode Pad.
The left and right Status Lights flash simultaneously.	Excessive current is flowing through the Device.	Check the transfer stack and ensure Gel Window covered correctly on the gel.
Inefficient transfer Empty spots on the membrane	1. Salt built-up on plate electrodes	1. Clean the titanium cathode plate, graphite anode plate with a wet cloth or paper tissue followed by a dry one to remove any insoluble salts.
	2. Membrane insufficiently equilibrated in eBlot® Equilibration buffer	2. Equilibrate membrane in eBlot® Equilibration buffer before transfer.
	3. Incorrect transfer conditions or insufficient transfer time	3. Use a gel of lower concentration to separate high molecular weight proteins. Increase the transfer time in 5-second increments.
	4. PVDF membrane was not prewet with methanol	4. Pre-wet PVDF membrane with methanol before transfer.
	5. Confusion of the eBlot® Anode Pad and Cathode Pad Air bubbles trapped between gel and membrane prevent the transfer of proteins	5. Ensure the transfer stack is assembled correctly: <i>Bottom-eBlot®</i> Anode Pad (yellow), <i>Top-eBlot®</i> Cathode Pad (white). When assembling transfer stack, use the small shovel supplied with the device to remove any air bubbles between the gel and the membrane