# eBlot<sup>™</sup> Protein Transfer System

#### November 19, 2012

Instructions for using the *e*Blot<sup>™</sup> Protein Transfer System to perform fast semi-dry electroblotting of proteins from mini polyacrylamide gels to membranes are described below. For detailed instructions, refer to the manual supplied with the product or download the manual from www.genscript.com.

## Assembling the Transfer Stack

1. Open the closed lid by pressing the **Open** button.



 Remove the eBlot<sup>™</sup> Anode Pad out of the package and place it on the anode plate.



- Soak the precut membrane in the *e*Blot<sup>™</sup> Equilibration Buffer for 1 minute. If PVDF membrane is used, pre-wet the membrane with methanol before equilibrating in *e*Blot<sup>™</sup> Equilibration Buffer.
- Place the membrane on the eBlot<sup>™</sup> Anode Pad. Gently remove air bubbles between the membrane and the anode pad using the small shovel supplied with the device.



5. Remove the pre-run gel containing your protein samples from gel cassette and briefly rinse the gel with distilled water.

6. Place the gel on the membrane and remove air bubbles between gel and pad using the small shovel supplied with the device.



7. Place appropriate Gel Window on the gel and ensure that the Gel Window fully cover the margin of the membrane.



8. Remove the *e*Blot<sup>™</sup> Cathode Pad from the package and place it on the gel.



9. Press the **Open** button, then push back and close the lid of the device.





## **Perform Blotting**

1. Press the **Min.** and **Sec.** buttons to set appropriate running time based on the protein size (see table below).

Protein size (kD)	Recommended running time (min)
<80	7
80-160	8-9
>160	10

Based on the initial results, you can increase or decrease the transfer time using the **Time** buttons in 5-second increments.

2. Press the **Start/Stop** button to activate the transfer. The running time begins to count down and right Status Light keeps flashing during the whole transfer program.



 Current automatically shuts off at the end of each run. The eBlot<sup>™</sup> Protein Transfer Device signals the end of blotting with repeated beeping sounds. Right Status Light stops flashing and the lower four digits show text (00:00). Press any button on the control panel to stop the beeping.

### Disassembling and Cleaning the eBlot<sup>™</sup> Device

- 1. Open the closed lid by pressing the **Open** button.
- 2. Carefully separate the transferred membrane from the transfer stack and proceed with further protein detection methods.
- 3. Discard the used *e*Blot<sup>™</sup> Protein Transfer Pad. Clean the titanium cathode, graphite anode and surrounding area with a dry cloth or paper towel.
- 4. Replace the Absorbent Filter Paper in the Waste Tray with a new one when it has soaked up the waste from 20 times of transfer.

#### **Replacing the Graphite Electrode**

 Press and hold **Min.** button for 2 seconds to toggle to numbering mode. Check using frequency of Graphite Electrode.



Switch Off the eBlot<sup>™</sup> Protein Transfer Device.
Open the lid and take the worn Graphite Electrode out of the device.



- Remove the new Graphite Electrode out of the package, and then place the new Graphite Electrode into the anode tank and close the lid of the device.
- Switch On the eBlot<sup>™</sup> Protein Transfer Device. Press and hold Min. button for 2 seconds to shift to numbering mode.When the upper three digits are flashing, press Reset button to zero the transferring times.



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