eBlot™

Protein Transfer System

For fast semi-dry electroblotting of proteins from mini polyacrylamide gels to nitrocellulose or PVDF membranes

Version No. 09192012



User Manual





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Table of Contents

Product Contents	1
Product Specifications	3
<i>e</i> Blot™ Protein Transfer Device	5
Accessory Products	7
Introduction	8
Quick Reference Guide	11
Protocols	12
Troubleshooting	21
Examples of Results	22
Technical Support	23
Warranty	24
Appendix	25

Product Contents

Type of products

This manual is supplied with the *e*Blot[™] Protein Transfer Device (L03010).

eBlot™ Protein Transfer

Device Contents

The contents of the <i>e</i> Blot [™] Protein Transfer Device are listed below:		
Component	Quantity	
eBlot™ Protein Transfer Device	1 each	
eBlot [™] Graphite Electrode (Installed inside the device)	1 each	
Sponge Cushion (Installed inside the device)	2 each	
Regional specific power cord	1 each	
Forceps	1 each	
Shovel	1 each	
Shallow Tray	2 each	

Upon Receiving the Instrument

Examine the unit carefully for any damage incurred during transit. File any damage claims with the carrier. The warranty does not cover in-transit damage.

eBlot[™] Protein Transfer

Pads

The following *e*Blot[™] Protein Transfer Pads are available from GenScript:

Product	Cat. No.
eBlot [™] Protein Transfer Pads	L03011
(Basic, without membrane, 20-pak)	
eBlot™ Protein Transfer Pads (Nitrocellulose, 20-pak)	L03013
eBlot™ Protein Transfer Pads (PVDF, 20-pak)	L03014

The eBlot[™] Protein Transfer Pads come with the following components:

Component	Basic	Nitrocellulose	PVDF
<i>e</i> Blot™ Protein Transfer Pad	20	20	20
Nitrocellulose membranes (20 sheets)	-	1	-
PVDF membranes (20 sheets)	-	-	1
eBlot™ Equilibration Buffer (125 ml)	2	2	2
Gel Window (66 mm×56 mm)	1	1	1
Gel Window (76 mm×64 mm)	1	1	1
Gel Window (88 mm×78 mm)	1	1	1
Absorbent Filter Paper	1	1	1
Sponge Cushion	2	2	2

Product Contents, continued

Components for *e*Blot[™] Protein Transfer Pad are as follows:

Component	Quantity
1× <i>e</i> Blot [™] Cathode Pad	1
1× <i>e</i> Blot [™] Anode Pad	1

Store the *e*Blot[™] Protein Transfer Pads at room temperature. For best results, use the *e*Blot[™] Protein Transfer Pads before the expiration date printed on the package.

eBlot™ Graphite Electrode

The eBlot™ Graphite Electrode (L03012) is available separately from GenScript:

Product	Cat. No.
eBlot™ Graphite Electrode	L03012

For best results, when a graphite electrode has been used for 100 times of protein transfer, replace it with a new one.

eBlot[™] Equilibration Buffer

itter	The eBlot™ Equilibration Buffer (M01078, 125 ml) is available separately from
	GenScript:

Product	Cat. No.
eBlot™ Equilibration Buffer	M01078

() Important

DO NOT start a run without properly assembled Transfer Stacks in place !

Product Specifications

Product Specifications, continued

Intended Use	For research use only. Not intended for human or animal diagnostic or therapeutic uses.			Gel Window	
				Inter frame size:	66 mm × 56 mm
<i>e</i> Blot™ Protein Transfer	Weight:	1.6 kg			76 mm × 64 mm
Device Specifications	Dimensions:	325 mm (l) ×195 mm (w) ×70 mm (h)		•• • • •	88 mm × 78 mm
	Electrical Parameters:	100-120 V, 220-240 V, 50/60 Hz, 3.2 A		Materials:	Polycarbonate
	Built-in Features:	Digital Display, Alarm, Light LED			
	Compatibility:	Suitable for fast electroblotting of proteins from mini		Absorbent Filter Paper Size:	80 mm × 70 mm
		polyacrylamide gels to PVDF or nitrocellulose membranes		Materials:	Vegetable fiber
	Materials:	Acrylonitrile Butadiene Styrene, Polycarbonate,		materials.	
		Aluminum, Titanium, Plasticized silicone.			
	Operating Temperature:	15 - 40 °C			
	Forceps:	Stainless steel			
	Shovel:	Polycarbonate			
	Shallow Tray:	Polycarbonate			
	Avoid acetone, dimethyl sulfoxide, and acetic acid. These reagents can erode		eBlot™ Graphite Electrode Specifications	The eBlot [™] Electrode is used as the replaceable anode electrode of eBlot [™] Protein Transfer Device and available separately from GenScript.	
	or domogo the dovice	or damage the device.		The specifications for $eBlot^{TM}$ Graphite Electrode are listed below:	
	or damage the device.			The specifications for eBlo	t [™] Graphite Electrode are listed below:
eBlot™ Protein Transfer		fer Pads are used with the <i>e</i> Blot™ Protein Transfer Device.		The specifications for <i>e</i> Blo	t [™] Graphite Electrode are listed below:
eBlot™ Protein Transfer Pads Specifications	The <i>e</i> Blot [™] Protein Transf	fer Pads are used with the <i>e</i> Blot™ Protein Transfer Device. Blot™ Protein Transfer Pads are listed below:		The specifications for <i>e</i> Blo eBlot™ Graphite Electroo	
	The <i>e</i> Blot [™] Protein Transf				
	The <i>e</i> Blot [™] Protein Transf	Blot™ Protein Transfer Pads are listed below:		eBlot™ Graphite Electroo	de
	The <i>e</i> Blot [™] Protein Transf The specifications of the <i>e</i>	Blot™ Protein Transfer Pads are listed below:		eBlot™ Graphite Electroo Dimensions:	de 105 mm (l) × 95 mm (w) × 10 mm (h)
	The eBlot [™] Protein Transf The specifications of the e eBlot™ Protein Transfer	Blot™ Protein Transfer Pads are listed below: Pad		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The <i>e</i> Blot [™] Protein Transf The specifications of the <i>e</i> <i>e</i> Blot [™] Protein Transfer <i>e</i> Blot [™] Anode Pad:	Blot™ Protein Transfer Pads are listed below: Pad 90 mm (I) × 80 mm (w) × 2.5 mm (thickness)		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad:	Blot™ Protein Transfer Pads are listed below: Pad 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) 90 mm (I) × 80 mm (w) × 2.5 mm (thickness)		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad:	Blot™ Protein Transfer Pads are listed below: Pad 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) Blotting filter paper presoaked with proprietary		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad: Materials:	Blot™ Protein Transfer Pads are listed below: Pad 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) Blotting filter paper presoaked with proprietary		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad: Materials:	Blot [™] Protein Transfer Pads are listed below: Pad 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) Blotting filter paper presoaked with proprietary anode or cathode buffer		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad: Materials: Membrane Nitrocellulose: PVDF:	Blot [™] Protein Transfer Pads are listed below: Pad 90 mm (l) × 80 mm (w) × 2.5 mm (thickness) 90 mm (l) × 80 mm (w) × 2.5 mm (thickness) Blotting filter paper presoaked with proprietary anode or cathode buffer 90 mm (l) × 80 mm (w) 90 mm (l) × 80 mm (w)		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad: Materials: Membrane Nitrocellulose:	Blot [™] Protein Transfer Pads are listed below: Pad 90 mm (l) × 80 mm (w) × 2.5 mm (thickness) 90 mm (l) × 80 mm (w) × 2.5 mm (thickness) Blotting filter paper presoaked with proprietary anode or cathode buffer 90 mm (l) × 80 mm (w) 90 mm (l) × 80 mm (w)		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g
	The eBlot [™] Protein Transf The specifications of the e eBlot [™] Protein Transfer eBlot [™] Anode Pad: eBlot [™] Cathode Pad: Materials: Membrane Nitrocellulose: PVDF: eBlot [™] Equilibration Buf	Blot™ Protein Transfer Pads are listed below: Pad 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) 90 mm (I) × 80 mm (w) × 2.5 mm (thickness) Blotting filter paper presoaked with proprietary anode or cathode buffer 90 mm (I) × 80 mm (w) 90 mm (I) × 80 mm (w) fer		eBlot™ Graphite Electroo Dimensions: Weight:	de 105 mm (I) × 95 mm (w) × 10 mm (h) 140 g

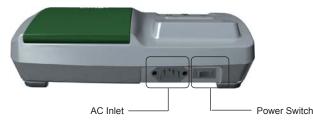
eBlot[™] Protein Transfer Device

Front View of eBlot™ Device

The front-top view showing various parts of the eBlotTM Protein Transfer Device is shown below.



Rear View of eBlot[™] Device The rear view showing various parts of the *e*Blot[™] Protein Transfer Device is shown below.



eBlot[™] Protein Transfer Device, continued

Control Panel of *e*Blot™ Device

The control panel of the eBlot[™] Protein Transfer Device is described below. The **Digital Display** shows two rows of multi-digits that specify the transfer conditions as follows:

The upper three digits after text <PN> indicate how many times of transfer the graphite electrode has been used for.

The lower four digits specify the running time of protein transfer in minute and second.

The two **Status Lights** show the working mode of the *e*Blot[™] Protein Transfer Device. When the right status light is on, the device is switched on and working at transferring mode; when both left and right status lights are on,

the device is working at numbering mode.

The **Reset** button is used to clear parameters. The **Min.** button is used to shift between transferring and numbering mode, and to set running time. Each short press will increase one minute. Each long press (2 seconds) will toggle working mode from transferring to numbering or the opposite. The **Sec.** button is also used to set running time, each press will increase 5 seconds. The **Start/Stop** button is used to activate/stop the transfer program.



Top View of Open *e*Blot™ Device The top view of open eBlot[™] Protein Transfer Device identifying various parts.



Accessory Products

Precast Gels and Premade	The precast Express™ PAGE Gels as well as premade buffers and buffer
Buffers	powders are available from GenScript. For details, contact Technical
	Support or visit www.genscript.com.

ONE-HOUR Western[™] Detection Kits

The ONE-HOUR Western[™] Detection Kits used for Western blotting analysis are available from GenScript. Ordering information is provided below. For more information, visit www.genscript.com or call Technical Support.

Product	Quantity	Cat. No.
ONE-HOUR Western [™] Basic Kit (Rabbit)	1 Kit (5 Assays)	L00204
ONE-HOUR Western™ Basic Kit (Mouse)	1 Kit (5 Assays)	L00205
ONE-HOUR Western [™] Basic Kit (Goat)	1 Kit (5 Assays)	L00399
ONE-HOUR Western [™] Standard Kit (Rabbit)	1 Kit (5 Assays)	L00204C
ONE-HOUR Western™ Standard Kit (Mouse)	1 Kit (5 Assays)	L00205C
ONE-HOUR Western [™] Standard Kit (Goat)	1 Kit (5 Assays)	L00228
ONE-HOUR Western [™] Standard Kit with TMB (Rabbit)	1 Kit (5 Assays)	L00204T
ONE-HOUR Western™ Standard Kit with TMB (Mouse)	1 Kit (5 Assays)	L00205T
ONE-HOUR Western [™] Standard Kit with TMB (Goat)	1 Kit (5 Assays)	L00228T
ONE-HOUR Western [™] Advanced Kit (Rabbit)	1 Kit (5 Assays)	L00241
ONE-HOUR Western™ AdvancedKit (Mouse)	1 Kit (5 Assays)	L00242
ONE-HOUR Western [™] Advanced Kit (Goat)	1 Kit (5 Assays)	L00243
ONE-HOUR IP-Western Kit (Rabbit)	1 Kit (5 Assays)	L00231
ONE-HOUR IP-Western Kit (Mouse)	1 Kit (5 Assays)	L00232
ONE-HOUR IP-Western Kit (Goat)	1 kit (5 Assays)	L00233
ONE-HOUR Western [™] Fluorescent Kit	1 Kit (10 Assays)	L00397
ONE-HOUR Western™ Multiplex Fluorescent Kit	1 Kit (10 Assays)	L00398

Introduction

System Overview	Semi-dry Western blotting is a common technique applied in protein research.
	Conventional semi-dry blotting is a cumbersome process, requiring time
	-consuming reagent preparation and setup, followed by an electrophoretic
	transfer that could take one hour or more. GenScript's eBlot™ Protein Transfer
	System accelerates the semi-dry blotting process without sacrificing performance.
	The eBlot [™] Protein Transfer System, consisting of the eBlot [™] Protein Transfer
	Device and <i>e</i> Blot [™] Protein Transfer Pads, enables researchers to quickly,
	reliably perform electrophoretic transfer of proteins from various types of mini
	polyacrylamide gels to membranes in 7 to 10 minutes without the need to
	prepare additional buffers. The proteins transferred using the $eBlot^{\text{TM}}$ Protein
	Transfer System exhibit high detection sensitivity as to proteins transferred
	using other existing blotting methods.
System Components	The <i>e</i> Blot™ Protein Transfer System consists of:
	eBlot™ Protein Transfer Device
	The $eBlot^{TM}$ Protein Transfer Device is a self-contained electroblotting unit
	with a new an all holds in that allows for fact transfer of motoins

with a power supply built-in that allows for fast transfer of proteins.

eBlot[™] Protein Transfer Pads

As the consumable part of *e*Blot[™] Protein Transfer System, the *e*Blot[™] Protein Transfer Pads are the mixed assortment of *e*Blot[™] Protein Transfer Pad, nitrocellulose or PVDF membranes, Equilibration Buffer and Gel Window. Each pack of *e*Blot[™] Protein Transfer Pad contains an 1×*e*Blot[™] Cathode Pad and an 1×*e*Blot[™] Anode Pad presoaked with proprietary cathode buffer and anode buffer respectively, allowing for rapid, convenient and reliable protein blotting without the need to prepare additional buffers.

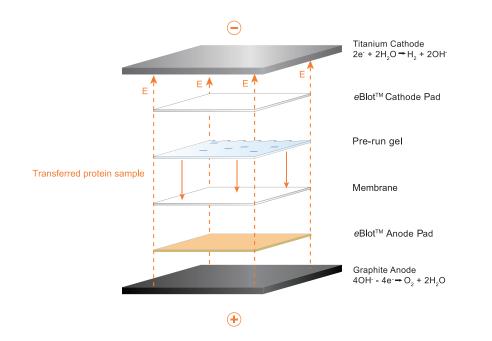
Introduction, continued

System Mechanism

The *e*Blot[™] Protein Transfer System is based on the proprietary fast semi-dry blotting technique developed by GenScript.

To use the eBlot[™] Protein Transfer System for rapid protein blotting, assemble the eBlot[™] Protein Transfer Pad with your pre-run gel and the membrane on the eBlot[™] Protein Transfer Device. The eBlot[™] Cathode Pad and eBlot[™] Anode Pad act as ion reservoirs that contain the appropriate anode and cathode buffers. The design of the eBlot[™] Protein Transfer Device reduces the distance between electrodes and the integrated power supply enables the system to generate a certain definite voltage allowing for rapid and directional movement of negatively charged protein molecules from the gel matrix onto the membrane within 7-10 minutes. Meanwhile, the membrane presoaked with *e*Blot[™] Equilibration Buffer shows good combination ability with proteins that prevents the flow through of low molecular weight proteins from the membrane.

Schematic mechanism of *e*Blot[™] Protein Transfer System showing the flow of current:



Introduction, continued

System Features

Important features of the *e*Blot[™] Protein Transfer System are listed below:

- Unique semi-dry electroblotting technique created for fast, reliable protein transfer within 7-10 minutes.
- Self-contained electroblotting unit with an integrated power supply for easy and convenient procedures.
- Consumable transfer pads offering convenience without the need for additional buffers.
- · Compatible for use with various types of mini polyacrylamide gels.
- · Proprietary formula without methanol.
- · High transfer efficiency as compared to other existing blotting methods.

Quick Reference Guide

Introc	duction

A quick reference guide for operating the *e*Blot[™] Protein Transfer Device is provided below.

Mode	Action	Sound	Light	Display
<i>e</i> Blot [™] Device	Connect <i>e</i> Blot™	-	Steady right	Default
plugged in	Device to an electrical		light	running
	outlet and power			time (00:00)
	switch is on			
<i>e</i> Blot [™] Device and	Assemble transfer	_	Steady right	Default
transfer stack	stack on the device		light	running
assembled	and close lid			time (00:00)
Time selection	Press Min. and Sec.	_	Steady right	User specified
	button to select		light	running
	desired running time			time (00:00)
Run	Press Start/Stop	-	Flashing right	Counting down
	button		light	time
End of run	Automatic	Continuous beeping	Steady right	Default running
		for 2 minutes	light	time (00:00)
Checking the number	Press and hold Min.	_	Steady left	Times of the
of uses of the	button for 2 seconds		and right lights	graphite anode
graphite anode				has been used
				for transfer
Replacement of	Switch off the device	_	_	_
worn graphite	and replace the worn			
anode	graphite anode with a			
	new one			

Protocols

Installing the

eBlot[™] Device

Recommendations	To obtain the best results, follow these recommendations:
	1. Wear gloves at all times during the entire blotting procedures to prevent
	contamination of pads, gels and membranes.
	2. Do not touch the gel or membrane with bare or gloved hands.
	This may contaminate the gel or membrane and interfere with further
	analysis. If needed, always use forceps to adjust the membrane or gel.

- Avoid using expired eBlot[™] Protein Transfer Pads and eBlot[™] Equilibration Buffer. Always use the pads and buffer before the specified expiration date printed on the package.
- Remove any trapped air bubbles between the gel and membrane during the assembly of the transfer stack using the small shovel supplied with the device.

 Check the Power Cord supplied with the unit to ensure that the cord is compatible with the local socket format.

- Place the eBlot[™] Protein Transfer Device on a levelled laboratory bench. Keep the area around the device clear to ensure proper ventilation of the unit.
- 3. For your safety: Position the device properly such that the Power Switch and the AC inlet located on the rear of the unit are easily accessible.
- 4. Ensure the Power Switch is in the Off position.
- Open the closed lid of the eBlot[™] Protein Transfer Device by pressing the **Open** button. Place one or two pieces of Sponge Cushion in the anode tank depending on gel thickness. For 1.5 mm gel, use one piece of Sponge Cushion; for 0.75 and 1.0 mm gel, use two pieces of Sponge Cushion.
- **Note:** After 20 times of protein transfer, replace the used Sponge Cushions with new ones. A pair of new Sponge Cushions are included in each box of eBlot[™] Protein Transfer Pads.
- Insert the eBlot[™] Graphite Electrode into the anode tank as described in Section "Replacing the eBlot[™] Graphite Electrode", then close the lid of the device.

Installing the eBlot[™] Device , continued Pull out the Waste Tray from the right side of the device. Place a new Absorbent Filter Paper inside the tray and then push the tray in.
 Note: Change absorbent paper with a new one when opening a new box of pads.



- Attach the power cord to the AC inlet and then to the electrical outlet. Use only properly grounded AC outlets and power cords.
- 9. When the electrophoresis of your samples is almost complete, press the Power Switch (located on the rear of the device) to turn **ON** the *e*Blot[™] Protein Transfer Device. The right Status Light is on indicating you are using transfer mode. The lower four digits of the Digital Display show the default running time (00:00).



You are ready to use the *e*Blot[™] Protein Transfer Device for blotting application.

Assembling the Transfer Stack

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



Protocols, continued

- Assembling the Transfer Stack, continued
- Remove one package labeled as eBlot[™] Protein Transfer Pad from the eBlot[™] Protein Transfer Pads box and tear the laminated sealing of the package. Remove the two small packages respectively labeled 1×eBlot[™] Cathode Pad and 1×eBlot[™] Anode Pad.



 Tear the sealing of the 1×eBlot[™] Anode Pad package. Remove the eBlot[™] Anode Pad from the package and place it on the anode plate of the eBlot[™] Protein Transfer Device.



- Pour 10 ml eBlot[™] Equilibration Buffer into the shallow tray supplied with the eBlot[™] Device.
- Tear the sealing of the Nitrocellulose or PVDF Membranes package. Remove one sheet of membrane and soak it in the *e*Blot[™] Equilibration Buffer for 1 minute.
- Note: If a PVDF membrane is used, the membrane must be pre-wetted with methanol before equilibrating in eBlot[™] Equilibration Buffer. For users of eBlot[™] Protein Transfer Pads (Basic, 20-pak) (L03011), the precut membrane should be prepared by themselves.

Assembling the Transfer Stack, continued

 Place the equilibrated membrane on the eBlot[™] Anode Pad. Gently remove air bubbles between the membrane and the anode pad using the small shovel supplied with the device.



- 7. Carefully remove the pre-run gel containing your protein samples from the gel cassette and briefly rinse the gel with distilled water in another shallow tray to remove any small gel pieces attached to the gel and facilitate easy positioning of the gel on the membrane.
- 8. Place the gel on the membrane. Gently remove air bubbles between the gel and the membrane.



Protocols, continued

Assembling the Transfer Stack, continued

 Select appropriate Gel Window according to actual size of the gel (see the table below). Place Gel Window on the gel. Ensure that the Gel Window fully covers the margin of the membrane.

Pre-run gel	Gel Window
Gel with size > 80 × 60 mm	Gel Window 88 × 78 mm
Gel with size 66 × 56 mm to 80 × 60 mm	Gel Window 76 × 64 mm
Gel with size < 66 × 56 mm	Gel Window 66 × 56 mm

Note: Gel Window is used as the spacer between anode pad and cathode pad to

prevent short circuit.



 Tear the sealing of the 1×eBlot[™] Cathode Pad package. Remove the eBlot[™] Cathode Pad from the package and place it on the gel.



Note: During assembling of the transfer stack, make sure to remove all the air bubbles trapped between the transfer pads, pre-run gel and the membrane, which may prevent the transfer of proteins and cause empty spots on the transferred membrane.

- 15 -

- Assembling the Transfer Stack, continued
 - Press the **Open** button, and then push back and close the lid of *e*Blot[™]
 Protein Transfer Device.
- Performing Blotting
 Perform protein blotting as described below within 15 minutes of assembling the transfer stack.
 - Press the Min. and Sec. buttons to set appropriate running time based on the protein size (see table below). If an undesired running time is set by mistake, press Reset button to clear the wrong time, and then press the Min. and Sec. buttons to choose the desired running time.



Protoin cizo (kDo)	Recommended		
Protein size (kDa)	start running time (min)		
< 80	7		
80-160	8-9		
> 160	10		

- Note: Based on the initial results, the transfer time may need to be optimized to make best transfer results by pressing the Time button in 5-second increment. For proteins greater than 200 kDa, we recommend to start with 11 minutes.
- Press the Start/Stop button to start the transfer. The running time begins to count down and right Status Light keeps flashing during the whole transfer program.



Protocols, continued

- Performing Blotting, continued
- At the end of the transfer, current automatically shuts off and the eBlot[™] Protein Transfer Device signals the end of transfer with repeated beeping sound. The right status light stops flashing and the lower five digits show text (00:00).
- 4. Press any button on the control panel to stop the beeping.
- 5. Proceed to disassemble the stack and clean the device.
- Disassembling and Cleaning the eBlot™ Device
- To obtain good transfer and detection results, disassemble the transfer stack right away after ending the blotting procedure.
- 1. Open the closed lid by pressing the **Open** button.
- Carefully separate the transferred membrane from the transfer stack and proceed with further protein detection procedures.
- **Note:** If you are using PVDF membrane, place the membrane immediately into the blocking solution (or water) as PVDF membrane dries quickly. If the PVDF membrane is dried, rewet the membrane with methanol and then rinse it with distilled water to wet it completely before use.
- 3. Discard the gel and the used *e*Blot[™] Protein Transfer Pad.
- **Note:** Do not re-use the eBlot[™] Protein Transfer Pad after blotting. Discard after each use.
- 4. Clean the titanium cathode plate, graphite anode plate and its surrounding area with a dry cloth or paper tissue.
- 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. Pull out the Waste Tray from the right side of the device to perform the replacement.
- **Note:** After about 40 runs, take out the graphite electrode, soak in distilled water for 30 minutes, dry the surface with a clean paper towel, then leave air dry overnight with graphite side up.

At this point, the *e*Blot[™] Protein Transfer Device is ready for another run. If you are not using the device, turn off the Power Switch located on the back of the device.

For any other repairs and service, contact Technical Support. Do not perform any repairs or service on the *e*Blot[™] Protein Transfer Device by yourself to avoid any possible damages to the device.

Replacing the Graphite Electrode

During the transfer process, the Graphite Electrode will absorb ions from anode pad as well as lose carbon composition of the anode buffer. For best blotting results, after having been used for **100 times** of transfer, the worn Graphite Electrode should be replaced by a new one.

 If the eBlot[™] Protein Transfer Device works at transferring mode, press and hold **Min.** button for 2 seconds to toggle to numbering mode. If the upper three digits show "100" or a number greater than "100", perform the replacing protocol as describe below.



 Switch Off the eBlot[™] Protein Transfer Device. Open the lid of the device and take the worn Graphite Electrode out of the device.



 Tear the sealing of a new Graphite Electrode package and take the new Graphite Electrode out of the package. Place the new Graphite Electrode into the anode tank and close the lid of the device.

Protocols, continued

- Replacing the Graphite Electrode, continued
- Switch On the eBlot[™] Device. Press and hold Min. button for 2 seconds to toggle to numbering mode. When the upper three digits are flashing, press Reset button to zero the transferring times.



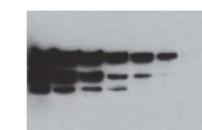
After successfully installing Graphite Electrode into the eBlotTM Device, you are ready to use the eBlotTM Device for another blotting application.

Troubleshooting

Problem	Cause	Solution
The right Status Light doesn't	Incomplete electric circuit due	Ensure the transfer stack is
flash during blotting process.	to improper assembly of the	assembled correctly: use the
	transfer pads.	<i>e</i> Blot [™] Anode Pad first followed
		by the membrane, the pre-run ge
		Gel Window and <i>e</i> Blot [™] Cathode
		Pad.
The left and right Status	Excessive current is flowing	Check the transfer stack and
Lights flash simultaneously.	through the Device.	ensure Gel Window covered
		correctly on the gel.
Inefficient transfer	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	2. Equilibrate membrane in
	equilibrated in <i>e</i> Blot [™] Equilibration	eBlot™ Equilibration
	buffer	buffer before transfer.
-	3. Incorrect transfer conditions or	3. Use a gel of lower
	insufficient transfer time	concentration to separate
		high molecular weight protein
		Increase the transfer time in
		5-second increments.
-	4. PVDF membrane was not prewet	4. Prewet PVDF membrane
	with methanol	with methonal before
		transfer.
-	5. Confusion of the <i>e</i> Blot [™] Anode	5. Ensure the transfer stack is
	Pad and Cathode Pad	assembled correctly:
		<i>Bottom-e</i> Blot [™] Anode Pad
		(yellow), Top- <i>e</i> Blot™ Cathode
		Pad (white).
Empty spots on the membrane	Air bubbles trapped between gel	When assembling transfer stack,
• •	and membrane prevent the	use the small shovel supplied wit
	transfer of proteins.	the device to remove any air
		bubbles between the gel and the
		membrane.

Examples of Results

Westen blot result after using the *e*Blot[™] for protein transfer



← α-Tubulin (55 kDa)
 ← β-Actin (42 kDa)
 ← GAPDH (36 kDa)

Hela cell lysate (µg) 10 5 2.5 1.25 0.62 0.31 0.16

An Express[™] PAGE Gels 8-16% (GenScript MG816W12) was blotted using the eBlot[™]. 10.0, 5.0, 2.5, 1.25, 0.62, 0.31 and 0.16 µg of HeLa cell lysate were loaded respectively. Proteins on the Nitrocellulose membrane were detected using THE[™] alpha Tubulin Antibody (GenScript, A01410), THE[™] beta Actin Antibody (GenScript, A00702) and GAPDH Antibody (GenScript, A01622). The secondary antibody was Anti-MOUSE IgG (H & L) (GOAT) Antibody Peroxidase Conjugated (Rockland, 610-1302). The signal was developed with LumiSensor[™] HRP Substrate Kit (GenScript, L00221V500)

Result Using Nitrocellulose

1	2	3	4	5	6	7	8	9
		-	-					
		-			-	-		
						-	-	-
-				-				
								-
			=	-	-	=	=	-
=	=	-				-		
April 1	-					1001	1000	

Good transfer of protein standard bands onto the nitrocellulose membrane. Protein Standards were separated on a 4-20% Genscript Express[™] Plus PAGE Gel (Bis-Tris). After electrophoresis, the gel was blotted using the *e*Blot[™] protein transfer system for 11 min as described in this manual. Lane 1 and 2, Invitrogen Pre-Stain protein marker (5 µL, MW: 4-250 kDa), Lane 3 and 4, Bio-rad Pre-Stain protein marker (10 µL, MW: 10-250 kDa), and Lane 5-9, NEB All Blue Pre-Stain protein marker (0.3, 0.6, 1.3, 2.5, and 5 µL, MW: 10-230 kDa).

Technical Support

Web Resources

Contact Us

Visit the GenScript Web site at www.genscript.com for:

- 1. Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, *etc.*
- 2. Complete technical support contact information
- 3. Access to the GenScript Online Catalog
- 4. Additional product information and special offers

Warranty

eBlot [™] Protein Transfer	GenScript warrants that eBlot [™] Protein Transfer Device will be free from
Device	defects in material and workmanship for a period of one year from date of
	purchase. If any defects occur in the product during this warranty period,
	GenScript will, at its option, repair, replace, or refund the purchase price of
	this product at no charge to you. The following defects, however, are
	specifically excluded:
	1. Defects caused by improper operation.
	2. Repair or modification done by anyone other than GenScript or an
	authorized agent.
	3. Use of fittings or other spare parts supplied by anyone other than
	GenScript.
	4. Damage caused by accident or misuse.
	5. Damage caused by disaster.
	6. Corrosion due to use of improper solvent or sample.
	For any inquiry or request for repair service, contact GenScript after
	confirming the model and serial number of your instrument. For your

confirming the model and serial number of your instrument. For your protection, items being returned must be insured against possible damage or loss. This warranty shall be limited to the replacement of defective products. It is expressly agreed that this warranty will be in lieu of all warranties of fitness and in lieu of the warranty of merchantability.

For more information or technical assistance, call, write, fax, or email.

GenScript USA Inc.

860 Centennial Ave. Piscataway, NJ 08854 Tel: 732-885-9188, 732-885-9688 Fax: 732-210-0262, 732-885-5878 Email: product@genscript.com

Appendix



Appendix, continued



Appendix, continued

	AI	ION OF CONFORMITY
. Ac	cor	ding to FCC Part 15B
Responsible Party's Name	:	Certificate No.: SEM11058591 NanJing Genscript Co., Ltd.
Address	:	No.78 Shuangbai Road, Xuanwu District, Nanjing, China
Manufacturer	:	NanJing Genscript Co., Ltd.
Address	:	No.78 Shuangbai Road, Xuanwu District, Nanjing, China
Description of Product	:	Multifunction Gel Processor
Model No.	:	GS-01, GS-02
Trade Name	:	eStain, eBlot
Report No.	:	STR11058051E-3
Compliance With Part 15B of	FCC	Rules.
		his device must accept any interference received, including interference cause undesired operation.
Responsible Party:		Tested By:
		=
		SEM.Test Compliance Service Co., Ltd.
		3/F, Jinbao Commerce Building, Xin'an Fanshen Road, Bao'an District, Shenzhen, P.R.C.
		Tompliance
Responsible Signature:		Issued By:
Name / Title:		Name / Title: Jandy So 7 PSQ Manager
Date:	-	Date of Issue: Jul 12, 2011 .
The Cartification of Verification show	s that	t the tested sample technically compliances with the FCC Part 15. T
the certification of vernication show		we mentioned only and should not implied an assessment of the whole.