GenScript
The Biology CRO

eStain™

Protein Staining System

For electric staining of proteins in mini polyacrylamide gels

Version 2.0

June 4, 2010

User Manual

Table of Contents

Product Contents

Type of products

This manual is supplied with the following products:

Product	Cat. No.
*e*Stain™ Protein Staining Device	L02010

*e*Stain™ Protein Staining Device Contents

The contents of the *e*Stain™ Protein Staining Device are listed below

Component	Quantity
*e*Stain™ Protein Staining Device	1 each
Specific Power Cord based on the type of unit ordered (for U.S./Canada/China/ Taiwan/Japan, Europe, or UK)	1 each
Forceps	1 each
Shovel	1 each
*e*Stain™ Graphite Electrode (11mm) (installed inside the device)	1 each
Ice Pack	1 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.

*e*Stain™ Protein Staining Pads

The following *e*Stain™ Protein Staining Pads are available from GenScript:

Product	Cat. No.
*e*Stain™ Protein Staining Pads (R-250, 20-pak)	L02011
*e*Stain™ Protein Staining Pads (G-250, 20-pak)	L02012

If you ordered the eStain™ Protein Staining Pads, you will receive the components listed in the table below. Store the eStain™ Protein Staining Pads at room temperature. For best results, use the eStain™ Staining Pads before the expiration date printed on the package.

Product Contents, continued

_e_Stain™ Protein Staining Pads, continued

Component	Quantity
The *e*Stain™ Protein Staining Pads (R-250, 20-pak) contains:	
*e*Stain™ Protein Staining Pad (R-250)	20
Gel Window (66mm×56mm)	1
Gel Window (76mm×64mm)	1
The *e*Stain™ Protein Staining Pads (G-250, 20-pak) contains:	
*e*Stain™ Protein Staining Pad (G-250)	20
Gel Window (66mm×56mm)	1
Gel Window (76mm×64mm)	1
each pack *e*Stain™ Protein Staining Pad (R-250) contains:	
1×*e*Stain™ Cathode Pad (R-250)	1
1×*e*Stain™ Anode Pad	1
each pack *e*Stain™ Protein Staining Pad (G-250) contains:	
1×*e*Stain™ Cathode Pad (G-250)	1
1×*e*Stain™ Anode Pad	1

_e_Stain™ Graphite Electrode

The following *e*Stain™ Graphite Electrode is available from GenScript:

Product	Cat. No.
*e*Stain™ Graphite Electrode (11mm, 1-pak)	L02014

For best results, when a Graphite Electrode was used for 200 times of electric staining, replace it with a new one.

Product Specifications

*e*Stain™ Protein Staining Device Specifications

Weight:	2.3 kg
Dimensions:	200 mm (l) ×180 mm (w) ×90 mm (h)
Electrical Parameters:	100-120 V, 220-240 V, 50/60 Hz, 3.2 A
Built-in Features:	Digital display, alarm, light LED
Compatibility:	Suitable for electric staining of proteins in mini polyacrylamide gels
Materials:	Acrylonitrile Butadiene Styrene, Polycarbonate, Aluminum, Titanium, Plasticized silicone.
Operating Temperature:	15 - 40°C
Forceps:	Stainless steel
Shovel:	Polycarbonate

Avoid acetone, dimethyl sulfoxide, and acetic acid. These reagents can erode or damage the device.

*e*Stain™ Protein Staining Pads Specifications

The *e*Stain™ Protein Staining Pads are used with the *e*Stain™ Protein Staining Device.

The specifications of the *e*Stain™ Protein Staining Pads are listed below:

_e_Stain™ Cathode Pad (R-250 or G-250)

size:	8cm (l) x 7cm (w) x2.5mm (thick)
Materials:	Blotting filter paper presoaked with proprietary cathode buffer containing CBB R-250 or G-250

_e_Stain™ Anode Pad

size:	8cm (l) x 7cm (w) x2.5mm (thick)
Materials:	Blotting filter paper presoaked with proprietary anode buffer

Gel Window

Inter frame size:	66mm×56mm 76mm×64mm
Materials:	Polycarbonate

Product Specifications, continued

*e*Stain™ Graphite Electrode Specifications

The *e*Stain™ Graphite Electrode is used as the replaceable anode of *e*Stain™ Protein Staining Device and available separately from GenScript.

The specifications for *e*Stain™ Graphite Electrode are listed below:

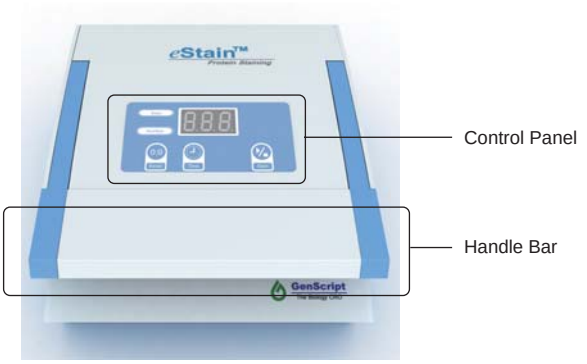
eStain™ Graphite Electrode (11mm)

Dimensions:	100 mm (l) ×90 mm (w) ×11mm (h)
Weight:	180g
Materials:	Powdered carbon, clay

*e*Stain™ Protein Staining Device

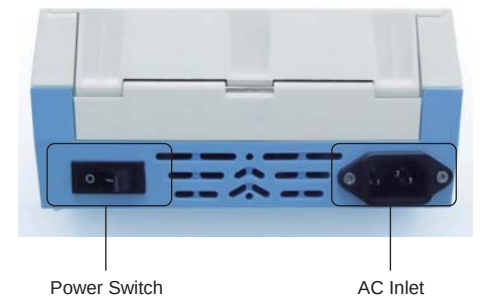
Front View of *e*Stain™ Device

The front top view showing various parts of the *e*Stain™ Protein Staining Device is shown below.



Rear View of *e*Stain™ Device

The rear view showing various parts of the *e*Stain™ Protein Staining Device is shown below.



*e*Stain™ Protein Staining Device, continued

Control Panel of *e*Stain™ Device

The control panel of the *e*Stain™ Protein Staining Device is described below. When upper status light is on, which indicates staining mode, the Digital Display shows running time; otherwise when both upper and lower status lights are on, which indicates numbering mode, the Digital Display shows how many times of electric staining the *e*Stain™ Graphite Electrode has been used for.

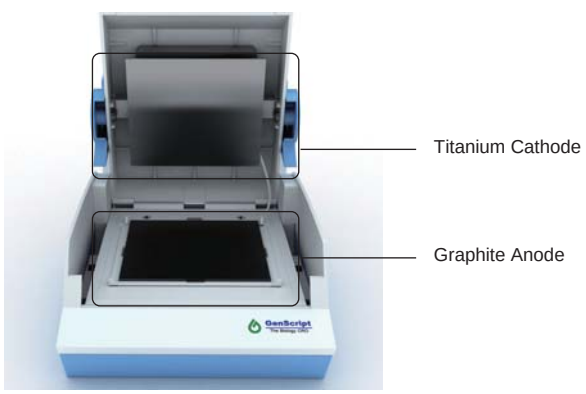
The **Reset** button is used to clear parameters.

The **Time** button is used to set running time and toggle between staining mode and numbering mode.

The **Start/Stop** button is used to activate/stop the staining program.

Inside View of *e*Stain™ Device

The inside view showing various parts of the *e*Stain™ Protein Staining Device is shown below:



Accessory Products

*e*Stain™ Protein Staining Pads

*e*Stain™ Protein Staining Pads are available separately from GenScript. Ordering information is provided below.

Product	Quantity	Cat. No.
*e*Stain™ Protein Staining Pads (R-250, 20-pak)	1 box	L02011
*e*Stain™ Protein Staining Pads (G-250, 20-pak)	1 box	L02012

*e*Stain™ Graphite Electrode

*e*Stain™ Graphite Electrode is available separately from GenScript. Ordering information is provided below.

Product	Quantity	Cat. No.
*e*Stain™ Graphite Electrode (11mm, 1-pak)	1 unit	L02014

Additional Products

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

Introduction

System Overview

The eStain™ Protein Staining System is a unique electric staining system that allows you to quickly, reliably and efficiently stain proteins in various types of mini polyacrylamide gels with Coomassie blue dye reagents. It consists of the eStain™ Protein Staining Device and eStain™ Protein Staining Pads.

The proprietary electric staining technology of the eStain™ Protein Staining Device combined with the eStain™ Protein Staining Pads applies a high electric force generated between graphite anode and titanium cathode to allow for quick and directional movement of negatively charged Coomassie blue dye reagents into the gel matrix to stain the proteins and also the quick and directional movement of the free staining reagents out of the gel matrix to destain the gel matrix within only 7 minutes or less. The eStain™ Protein Staining System integrates three steps of conventional Coomassie blue staining method into a single step and greatly cuts down the time required for protein staining analysis. The proteins stained using the eStain™ Protein Staining System exhibit as high detection sensitivity as proteins stained using conventional Coomassie blue staining method.

System Components

The eStain™ Protein Staining System consists of:

eStain™ Protein Staining Device

The eStain™ Protein Staining Device is a user-friendly electric staining unit that allows for fast, convenient and efficient in-gel protein staining with Coomassie blue dye reagents.

eStain™ Protein Staining Pads

The eStain™ Protein Staining Pads are disposable pads to perform electric staining of proteins in gels with Coomassie blue dye. Two types of eStain™ Protein Staining Pad, R-250 and G-250, are available separately from GenScript and for different requirements. Each pack eStain™ Protein Staining Pad contains an eStain™ Cathode Pad presoaked with proprietary cathode buffer containing CBB dye R-250 or G-250, and an eStain™ Anode Pad presoaked with proprietary anode buffer, allowing for rapid, convenient and reliable in-gel protein staining without the need to prepare any solutions and buffers.

Introduction, continued

System Components, continued

***e*Stain™ Graphite Electrode**

The *e*Stain™ Graphite Electrode serves as the replaceable anode of the *e*Stain™ Protein Staining Device. For the common use, one *e*Stain™ Graphite Electrode (11mm) is pre-installed inside the *e*Stain™ Protein Staining Device to constitute a complete electric staining unit. Each *e*Stain™ Graphite Electrode is produced with special process and can be steadily used for 200 times of electric staining.

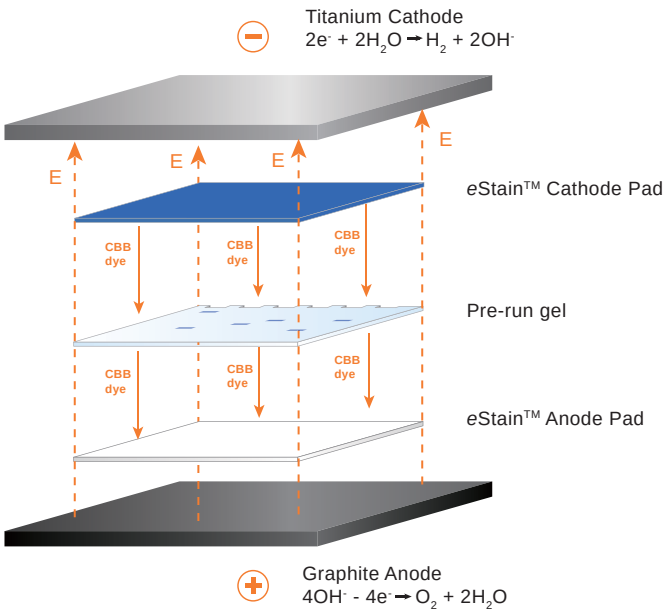
System Mechanism

The *e*Stain™ Protein Staining System is based on the electric staining concept which utilizes the unique and patent-pending technology developed by GenScript. To use the *e*Stain™ Protein Staining System for in-gel protein staining, assemble the *e*Stain™ Protein Staining Pad with your pre-run gel on the *e*Stain™ Protein Staining Device. Similar to semi-dry blotting, the *e*Stain™ Cathode Pad and *e*Stain™ Anode Pad act as ion reservoirs that contain the appropriate anode and cathode buffers incorporated into the gel matrix. Simultaneously the *e*Stain™ Cathode Pad presoaked with proprietary cathode buffer containing CBB dye R-250 or G250, also supplies negatively charged staining reagents. The high electric force generated between graphite anode and titanium cathode allows for rapid and directional movement of negatively charged Coomassie blue dye reagents into the gel matrix to stain the proteins and also the homogenous movement of the free staining reagents out of the gel matrix to destain the gel matrix within only 7 minutes or less. Therefore the *e*Stain™ Protein Staining System provides fast, convenient and reliable in-gel protein staining without the need to prepare any solutions and buffers.

Introduction, continued

System Mechanism, continued

Schematic mechanism of *e*Stain™ Protein Staining System showing the movement of Coomassie blue dye:



Features

Important features of the *e*Stain™ Protein Staining System are listed below:

- Unique electric staining technology created for fast, reliable and efficient protein gel staining in as few as 7 minutes.
- User-friendly electric staining unit allowing for easy and convenient procedures.
- Consumable staining pads offering convenience without the need for additional solutions and buffers.
- High staining efficiency as conventional Coomassie blue staining method.
- Compatible for use with various types of mini polyacrylamide gels.

Quick Reference Guide

Introduction

A quick reference guide for operating the *e*Stain™ Protein Staining Device is provided below.

Mode	Action	Sound	Light	Display
*e*Stain™ plugged in	Connect *e*Stain™ Device to an electrical outlet and power switch is on	–	Steady upper light	Default running time (000)
*e*Stain™ device and staining pad assembled	Place staining pads on the device and close lid	–	Steady upper light	Default running time (000)
Time selection	Press **Time** button to select desired running time	–	Steady upper light	Running time defined
Run	Press **Start/Stop** button	–	flashing upper light	Count down time
End of run	Automatic	Continuous beeping for 2 minutes	Steady upper light	Default running time (000)
Checking using frequency of graphite anode	Press and hold **Time** button for 2 seconds	–	Steady upper and lower light	Staining times the graphite anode has been used for
Replacement of worn graphite anode	Switch off the device and replace the worn graphite anode with a new one	–	–	–

Protocols

Recommendations

To obtain the best results follow these recommendations:

- Wear gloves at all times during the entire staining procedures to prevent contamination of pads and gels.
- Avoid using expired *e*Stain™ Protein Staining Pads. Always use the pads before the specified expiration date printed on the package.
- Always keep the Ice Pack supplied with *e*Stain™ Device stored at -20℃ or below to facilitate cooling electrodes procedure.

Installing the *e*Stain™ Device

1. Check the Power Cord supplied with the unit to ensure that the cord is compatible with the local socket format.
2. Place the *e*Stain™ Protein Staining Device on a level 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 AC power switch is in the **Off** position.
5. Attach the power cord to the AC inlet and then to the electrical outlet. Use only properly grounded AC outlets and power cords.
6. 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*Stain™ Protein Staining Device. The upper status light is on indicating you are using staining mode. Digital Display shows the default parameters (000).

You are ready to use the *e*Stain™ Protein Staining Device for staining applications.

Protocols, continued

Assembling Staining Stack with *e*Stain™ Pads and Pre-run Gel

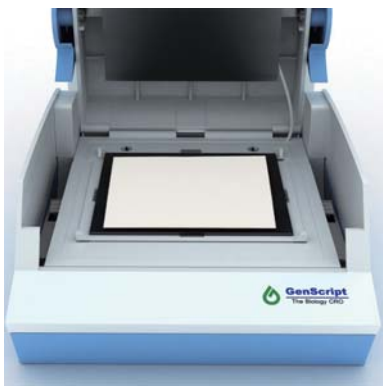
1. Lift up the handle bar to vertical position, then push it forward to open the lid of *e*Stain™ Device.

2. Remove one package labeled as *e*Stain™ Protein Staining Pad from the *e*Stain™ Protein Staining Pads box and tear the laminated sealing of the package. Remove the two small packages respectively labeled as 1 x *e*Stain™ Cathode Pad R-250 (or G-250), and 1 x *e*Stain™ Anode Pad.

Protocols, continued

Assembling Staining Stack with *e*Stain™ Pads and Pre-run Gel, continued

3. Tear the sealing of the 1 x *e*Stain™ Anode Pad package. Remove the *e*Stain™ Anode Pad from the package and place it on the anode plate of the *e*Stain™ Protein Staining Device.



4. Carefully remove the pre-run gel containing your protein samples from the gel cassette and rinse the gel with 50 to 100 ml distilled water for about 1 minute.

5. Place the gel on the *e*Stain™ Anode Pad. Gently remove air bubbles between gel and anode pad using the small shovel supplied with the device.

Protocols, continued

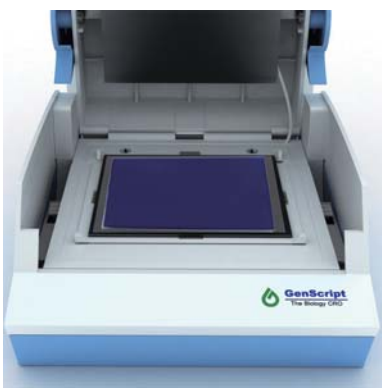
Assembling Staining Stack with *e*Stain™ Pads and Pre-run Gel, continued

6. 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 cover the margin of the anode pad.

Pre-run gel	Gel Window
Gel with size 66 x 56mm to 80 x 60 mm	Gel Windows 76 x 64mm
Gel with size <66 x 56 mm	Gel Windows 66 x 56mm

Note: *Gel Window is used as the spacer between anode pad and cathode pad to prevent short circuit. The edges of the pre-run gel may also be covered by Gel Window and will probably be stained to deep blue after running an electric staining program.*

7. Tear the sealing of the 1 x *e*Stain™ Cathode Pad package. Remove the *e*Stain™ Cathode Pad from the package and place it on the gel.



8. Pull back the handle bar to close the lid of *e*Stain™ Device while keeping the bar vertical to the lid, and then return the bar to horizontal position.

Protocols, continued

Performing electric staining

After assembling the staining stack, perform electric staining as described below.

1. Press the **Time** button to set appropriate running time based on the gel thickness (see table below). If an undesired running time is set by mistake, press **Reset** button to clear the wrong time, and then press again the **Time** button to choose the desired running time.

Gel Thickness	Running Time
0.75 mm	6 minutes
1.0 mm	7 minutes
1.5 mm	9 minutes

Note: *During electric staining process, the graphite electrode may be heated to 60-70℃. For continuous use and best results, it is necessary to cool down both graphite anode and titanium cathode to room temperature before the next run.*
(see next page for cooling procedures)

2. Press the **Start/Stop** button to activate the electric staining program. The running time begins to count down and upper status light keeps flashing during the whole staining program.

Protocols, continued

Performing electric staining, continued

3. At the end of the staining, current automatically shuts off and the *e*Stain™ Protein Staining Device signals the end of staining with repeated beeping sounds. Upper status light stops flashing and the Digital Display shows text (000).
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 *e*Stain™ Device

To obtain good staining results, disassemble the staining stack right away after ending the staining procedure.

1. Open the lid of *e*Stain™ Protein Staining Device.
2. Carefully separate the stained gel from the staining stack and proceed to be photographed.
3. Discard the used *e*Stain™ Protein Staining Pad.
   Note: *Do not re-use the eStain™ Protein Staining Pad after staining. Discard after each use.*
4. Clean the titanium cathode plate, graphite anode plate and its surrounding area with a dry cloth or paper towel.

Cooling Graphite Anode and Titanium Cathode

For continuous use and best results, it is necessary to cool down both graphite anode and titanium cathode to room temperature before the next run. Place the pre-frozen ice pack on the graphite anode, close the lid gently to let the titanium cathode also touches the ice pack. Do not force to close the lid otherwise the ice pack will damage the device.

At this point, the *e*Stain™ Protein Staining 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.

Protocols, continued

Replacing the *e*Stain™ Graphite Electrode

During electric staining process, the *e*Stain™ Graphite Electrode will absorb ions from anode pad and as well as lose carbon composition, thereby changing the characteristics of the Graphite Electrode and composition of the anode buffer. For the best staining results, after having been used for **200 times** of electric staining, the worn Graphite Electrode should be replaced by a new one.

1. If the *e*Stain™ Protein Staining Device works at staining mode, press and hold **Time** button for 2 seconds to toggle to numbering mode. If the Digital Display shows “200” or a number greater than 200, perform the replacing protocol as describe below.

2. Switch **Off** the *e*Stain™ Protein Staining Device and then open the lid of the device.

3. Use small shovel to pry out the worn Graphite Electrode along the gap at either side of the anode tank.

Protocols, continued

Replacing the *e*Stain™ Graphite Electrode, continued

4. Tear the sealing of a new *e*Stain™ 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.

5. Switch **On** the *e*Stain™ Device. Press and hold **Time** button for 2 seconds to toggle to numbering mode.

Protocols, continued

Replacing the *e*Stain™ Graphite Electrode, continued

6. Press **Reset** button to zero the staining times.

After successfully installing Graphite Electrode into the *e*Stain™ Device, you are ready to use the *e*Stain™ Device for staining applications again.

Note: *For the best results, after using Graphite Electrode per 40 times of electric staining, you may also need to perform a washing step by soaking the Graphite Electrode in distilled water for half an hour and then drying it with a dry cloth or paper towel.*

Troubleshooting

Introduction

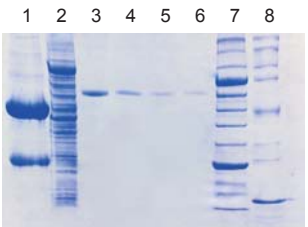
Review the information below to troubleshoot your experiments using the eStain™ Protein Staining Device and eStain™ Protein Staining Pad.

Problem	Cause	Solution
The upper status light doesn't flash during electric staining process	Incomplete electric circuit due to improper assembly of the staining pads.	Ensure the staining stack is assembled correctly: use the eStain™ Anode Pad first followed by the gel, Gel Window and eStain™ Cathode Pad.
The upper and lower status lights flash simultaneously	Excessive current is flowing through the Device.	Check the staining stack and ensure Gel Window covered correctly on the gel.
The stained gel has very tinged or nearly invisible protein bands with high blue background.	1. The staining time is not long enough. 2. Cooling the electrodes for too long a time. 3. The graphite electrode has been used for 200 times or above.	1. Cover Gel Window and the eStain™ Cathode Pad again and perform electric staining for 2 more minutes. 2. Follow the instruction to perform cooling procedure. 3. Replace the worn graphite electrode using a new one.
The stained gel has very tinged or nearly invisible protein bands with clear background.	1. The staining time is too long 2. The electrodes have not been cooled or the cooling time is not long enough	1. Re-assemble the staining stack using a new set of eStain™ Protein Staining Pad and then perform another 7-min electric staining. 2. Follow the instruction to perform cooling procedure.
The stained gel has hazy blue spots.	1. The gel has not been destained sufficiently. 2. Cooling the electrodes for too long a time 3. Air bubbles present in staining stack interfering gel staining.	1. Cover Gel Window and the eStain™ Cathode Pad again and perform electric staining for 1 more minute. 2. Follow the instruction to perform cooling procedure. 3. Be sure to remove all air bubbles present in the staining stack by using the small shovel supplied with eStain™ Device

Examples of Results

Tris-Glycine gel results

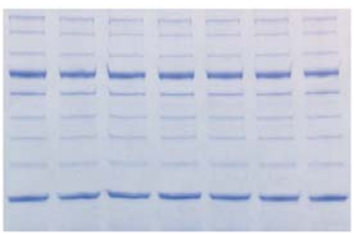
Samples were separated on a 12% Tris-Glycine gel and stained using eStain™ Protein Staining System (R-250).



Lane 1	500ng rabbit IgG
Lane 2	10μl *E.coli* lysate
Lane 3	200ng reduced BSA
Lane 4	125ng reduced BSA
Lane 5	50ng reduced BSA
Lane 6	15ng reduced BSA
Lane 7	10μl New England Biolabs® Protein Ladder (Cat. No. P7703S)
Lane 8	10μl GenScript Smart Middle Range Protein Standard (Cat. No. MM0900)

Bis-Tris gel results

Samples of 5μl New England Biolabs® Protein Ladder (P7703S) in each lane were separated on a 4-20% Bis-Tris gel and stained using eStain™ Protein Staining System (R-250).



Technical Support

Web Resources

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

Contact Us

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

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Warranty

eStain™ Protein Staining Device

GenScript warrants that eStain™ Protein Staining Device will be free from 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 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.

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