

eStain® 2.0

Protein Staining System

For fast Coomassie blue staining of proteins in mini polyacrylamide gels

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User Manual



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Product Contents

Type of Products

This manual is supplied with the following product:

Product	Cat. No.
eStain® 2.0 Protein Staining Device	L02016

eStain® 2.0 Protein Staining Device Contents

The contents of the eStain® 2.0 Protein Staining Device are listed below:

Component	Quantity
eStain® 2.0 Protein Staining Device	1 each
eStain® 2.0 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
Tray	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.

eStain® Protein Staining Pads

The following eStain® Protein Staining Pads are available from GenScript:

Product	Cat. No.
eStain® Protein Staining Pads (R-250, 20-pak)	L02011
eStain® Protein Staining Pads (G-250, 20-pak)	L02012

If you order 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® Protein Staining Pads before the expiration date printed on the package.

Product Contents, continued

eStain® Protein Staining Pads, continued

Component	Quantity
The eStain® Protein Staining Pads (R-250, 20-pak) contains:	
eStain® Protein Staining Pad (R-250)	20
Absorbent Filter Paper	1
Sponge Cushion	2
The eStain® Protein Staining Pads (G-250, 20-pak) contains:	
eStain® Protein Staining Pad (G-250)	20
Absorbent Filter Paper	1
Sponge Cushion	2
each pack of eStain® Protein Staining Pad (R-250) contains:	
1×eStain® Cathode Pad (R-250)	1
1×eStain® Anode Pad	1
each pack of eStain® Protein Staining Pad (G-250) contains:	
1×eStain® Cathode Pad (G-250)	1
1×eStain® Anode Pad	1

eStain® 2.0 Graphite Electrode

The following eStain® 2.0 Graphite Electrode is available from GenScript:

Product	Cat. No.
eStain® 2.0 Graphite Electrode	L02017

For best results, after 100 uses of electric staining, replace the worn eStain® 2.0 Graphite Electrode with a new one.

Product Specifications

eStain® 2.0 Protein Staining

Device Specifications

Weight:	1.78 kg
Dimensions:	325 mm (l) x 195 mm (w) x 70 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 fast Coomassie blue staining of proteins in mini polyacrylamide gels
Materials:	Acrylonitrile butadiene styrene, Polycarbonate, Aluminum, Titanium, Plasticized silicone.
Operating Temperature:	Room temperature
Forceps:	Stainless steel
Shovel:	Polycarbonate
Tray:	Polycarbonate

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

eStain® Protein Staining Pads Specifications

The eStain® Protein Staining Pads are used with the eStain® 2.0 Protein Staining Device.

The specifications of the eStain® Protein Staining Pads are listed below:

eStain® Cathode Pad (R-250 or G-250)

size:	90 mm (l) x 80 mm (w) x 2.5 mm (t)
Materials:	Blotting filter paper presoaked with proprietary cathode buffer containing CBB dye R-250 or G-250

eStain® Anode Pad

size:	90 mm (l) x 80 mm (w) x 2.5 mm (t)
Materials:	Blotting filter paper presoaked with proprietary anode buffer

Absorbent Filter Paper

Size:	80 mm (l) x 70 mm (w) x 2.6 mm (t)
Materials:	Vegetable fiber

Product Specifications, continued

eStain® Protein Staining

Pads Specifications, continued

Sponge Cushion

Size:	104 mm (l) x 100 mm (w) x 5 mm (t)
Materials:	Sponge

eStain® 2.0 Graphite Electrode Specifications

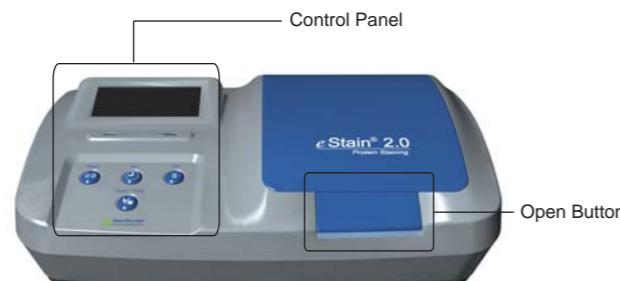
The eStain® 2.0 Graphite Electrode is used as the replaceable anode electrode of eStain® 2.0 Protein Staining Device and available separately from GenScript. The specifications for eStain® 2.0 Graphite Electrode are listed below:

Dimensions:	100 mm (l) x 92 mm (w) x 8.8 mm (t)
Weight:	206 g
Materials:	Powdered carbon, Clay, Stainless steel

eStain® 2.0 Protein Staining Device

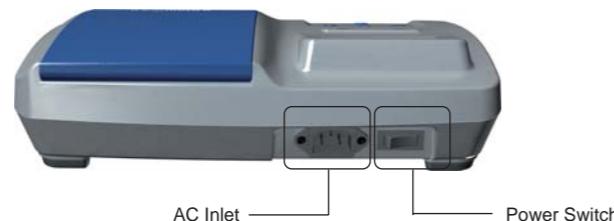
Front View of eStain® 2.0 Device

The front top view showing various parts of the eStain® 2.0 Protein Staining Device is shown below.



Rear View of eStain® 2.0 Device

The rear view showing various parts of the eStain® 2.0 Protein Staining Device is shown below.



eStain® 2.0 Protein Staining Device, continued

Control Panel of eStain® 2.0 Device

The control panel of the eStain® 2.0 Protein Staining Device is described below.

The **Digital Display** shows two rows of multi-digits that specify the electric staining conditions as follows:

The upper three digits after text <PN> indicate number of usage of the eStain® 2.0 Graphite Electrode.

The lower four digits specify the time of electric staining in minute and second.

The two status lights show the working modes of the eStain® 2.0 Protein Staining Device. When the right status light is on, the device is switched on and working at staining 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 staining 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 staining to numbering or vice versa.

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 staining program.



Top View of Openned eStain® 2.0 Device

The top view of opened eStain® 2.0 Protein Staining Device identifying various parts is shown below.



Introduction

System Overview

The eStain® 2.0 Protein Staining System, applying GenScript's patent-pending electric staining technique, allows you to quickly, reliably and efficiently stain proteins in various types of mini polyacrylamide gels with Coomassie blue dye. It consists of the eStain® 2.0 Protein Staining Device and the eStain® Protein Staining Pads. The proprietary electric staining technology of the eStain® 2.0 Protein Staining Device combined with the eStain® Protein Staining Pads applies a voltage generated between graphite anode and titanium cathode to allow for quick and directional movement of negatively charged Coomassie blue dye into the gel matrix to stain the proteins and also the homogeneous movement of the unbound staining reagents out of the gel matrix to destain the gel matrix within only 7 minutes or less. The eStain® 2.0 Protein Staining System integrates three steps of conventional Coomassie blue staining method into a single one and greatly cuts down the time required for protein staining analysis. The eStain® 2.0 Protein Staining System is able to deliver high detection sensitivity down to several ng per protein band, which is comparable to the sensitivity of traditional method despite of their different working principles.

System Components

The eStain® 2.0 Protein Staining System consists of:

eStain® 2.0 Protein Staining Device

The eStain® 2.0 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 disposable eStain® Protein Staining Pads are used to perform electric staining of proteins in gels with Coomassie blue dye. Two types of eStain® Protein Staining Pads, R-250 and G-250, are available separately from GenScript and for different requirements. Each pack of 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 additional buffers.

Introduction, continued

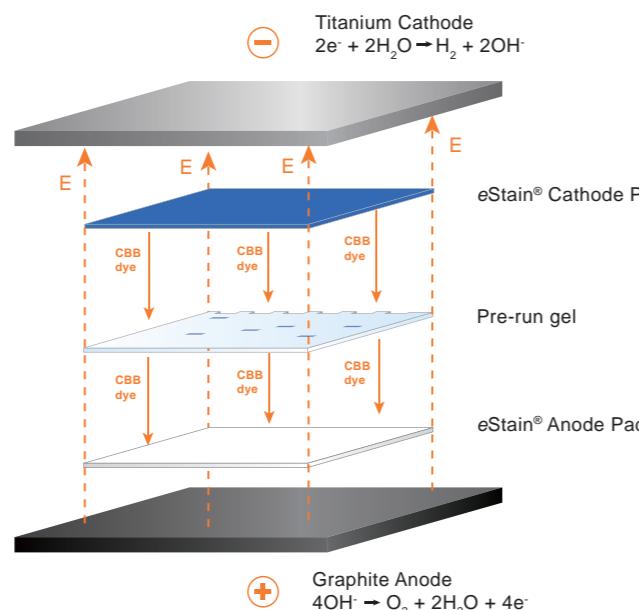
System Mechanism

The eStain® 2.0 Protein Staining System is based on GenScript's patent-pending electric staining technology. To use the eStain® 2.0 Protein Staining System for protein gel staining, assemble the eStain® Protein Staining Pad with your pre-run gel on the eStain® 2.0 Protein Staining Device. Similar to semi-dry blotting, the eStain® Cathode Pad and eStain® Anode Pad act as ion reservoirs with proprietary anode and cathode buffers. eStain® Cathode Pad, presoaked with cathode buffer containing CBB dye R-250 or G250, supplies negatively charged staining reagents. The voltage generated between graphite anode and titanium cathode allows for rapid and directional movement of negatively charged Coomassie blue dye into the gel matrix to stain proteins as well as for the homogeneous movement of the unbound staining reagents out of the gel matrix to destain the gel within only 7 minutes or less. Therefore the eStain® 2.0 Protein Staining System provides fast, convenient and reliable in-gel protein staining without the need to prepare additional buffers.

Introduction, continued

System Mechanism, continued

Schematic mechanism of eStain® 2.0 Protein Staining System showing the movement of Coomassie blue dye:



System Features

Important features of the eStain® 2.0 Protein Staining System are listed below:

- Innovative electric staining technology created for fast, reliable and efficient protein gel staining in 7 minutes or less.
- User-friendly electric staining unit allowing for easy and convenient procedures.
- Consumable staining pads offering convenience without the need for additional 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 eStain® 2.0 Protein Staining Device is provided below.

Mode	Action	Sound	Light	Display
Sponge cushion and graphite electrode installed inside eStain® 2.0 Device	Insert the sponge cushion and graphite electrode in the anode tank	–	–	–
eStain® 2.0 Device plugged in	Connect eStain® 2.0 Device to an electrical outlet and power switch is on	–	Steady right light	Default running time (00:00)
eStain® 2.0 Device and staining stack assembled	Place staining stack on the device and close lid	–	Steady right light	Default running time (00:00)
Time selection	Press Min. and Sec. button to select desired running time	–	Steady right light	Running time defined
Run	Press Start/Stop button	–	flashing right light	Count down time
End of run	Automatic	Continuous beeping for 2 minutes	Steady right light	Default running time (00:00)
Checking number of usage of graphite anode	Press and hold Min. button for 2 seconds	–	Steady left and right lights	Number of usage of the graphite electrode
Replacement of worn graphite anode	Switch off the device and replace the worn graphite anode with a new one	–	–	–

Protocols

Recommendations

Follow these recommendations for best results:

1. Wear gloves at all times during the entire staining procedures to prevent contamination of pads and gels.
2. Avoid using expired eStain® Protein Staining Pads. Always use the pads before the specified expiration date printed on the package.

Installing the eStain® 2.0 Device

1. Check the Power Cord supplied with the unit to ensure that the cord is compatible with local socket format.
2. Place the eStain® 2.0 Protein Staining Device on a leveled 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 that the AC power switch is in the **Off** position.
5. Open the closed lid of the eStain® 2.0 Protein Staining 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 electric staining, replace the used Sponge Cushions with new ones.

A pair of new Sponge Cushions are included in each box of eStain® Protein Staining Pads.

6. Insert the eStain® 2.0 Graphite Electrode into the anode tank as described in Section "Replacing the eStain® 2.0 Graphite Electrode", then close the lid of the device.

Protocols, continued

Installing the eStain® 2.0 Device, continued

Note: For a new eStain® 2.0 Protein Staining Device, two pieces of Sponge Cushion and one unit of eStain® 2.0 Graphite Electrode have already been installed inside the unit for immediate use. If staining 1.5 mm gel, remove 1 piece of Sponge Cushion.

7. 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 back in.

Note: After 20 times of electric staining, replace the used Absorbent Filter Paper with a new one. One piece of new Absorbent Filter Paper is included in each box of eStain® Protein Staining Pads.



8. Attach the power cord to the AC inlet, then, to the electrical outlet. Use only properly grounded AC outlets and power cords.
9. When the electrophoresis of your samples is almost completed, press the power switch (located on the rear of the device) to turn **ON** the eStain® 2.0 Protein Staining Device. The right status light is on indicating staining mode. The lower four digits of the digital display show the default running time (00:00).



You are ready to use the eStain® 2.0 Protein Staining Device for staining applications.

Protocols, continued

Assembling Staining Stack with eStain® Pads and Pre-run Gel

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



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



3. Tear the sealing of the 1×eStain® Anode Pad package. Remove the eStain® Anode Pad from the package and place it on the anode plate of the eStain® 2.0 Protein Staining Device.



4. Carefully remove the pre-run gel containing your protein samples from the gel cassette and briefly rinse the gel with distilled water.

Protocols, continued

Assembling Staining Stack with eStain® Pads and Pre-run Gel, continued

5. Place the gel on the eStain® Anode Pad. Gently remove air bubbles between gel and anode pad using the small shovel supplied with the device.



6. Tear the sealing of the 1×eStain® Cathode Pad package. Remove the eStain® Cathode Pad from the package and place it on top of the gel.



7. Press the **Open** button, then push back and close the lid of eStain® 2.0 Protein Staining Device.



Protocols, continued

Performing Electric Staining

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

Perform electric staining within 15 minutes after assembling the pads with the gel.

1. Use the following parameters as starting points for electric staining based on gel thickness.

Gel Thickness	Running Time
0.75 mm	6 - 7 minutes
1.0 mm	7 - 8 minutes
1.5 mm	7 - 8 minutes

Press the **Min.** and **Sec.** buttons to set appropriate running time. If an undesired running time is set by mistake, press **Reset** button to clear the wrong time, and then press again the **Min.** and **Sec.** buttons to choose the desired running time.



Note: For best staining results, it is recommended to use eStain® 2.0 Protein Staining System at room temperature (22 - 28 °C). If the ambient temperature is below 22 °C, you may need to extend the running time to obtain satisfied staining results based on gel thickness.

2. Press the **Start/Stop** button to activate the electric staining program. The running time begins to count down and right 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 eStain® 2.0 Protein Staining Device signals the end of staining with repeated beeping sounds. The right status light stops flashing and the lower four 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 eStain® 2.0 Device

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

1. Open the closed lid by pressing the **Open** button.
2. Carefully separate the stained gel from the staining stack and proceed to document the gel image.
3. Discard the used eStain® Protein Staining Pad.

Note: Do *Not* reuse the eStain® Protein Staining Pad. Discard after each use.

4. Take the eStain® 2.0 Graphite Electrode out of the device, dry the contact rods with a dry cloth or tissue paper. Take care not to lose the spring on the rod located near the triangle symbols before placing back into the device.

Caution: Do *Not* clean the eStain® 2.0 Graphite Electrode immediately after ending the staining program since the graphite electrode is still hot after a run. Wait for 3 to 5 minutes before proceeding to cleaning.

5. Clean the titanium cathode and the anode tank with a dry cloth or tissue paper.
6. Replace the used Sponge Cushions with new ones after 20 times of electric staining.
7. Replace the used Absorbent Filter Paper in the waste tray with a new one after 20 times of electric staining.

At this point, the eStain® 2.0 Protein Staining Device is ready for another run. If you are not using the device for a period of time, turn off the power switch located on the back of the device, take the graphite anode out, and keep the lid open to let any solution left in the holes for the contact rods dry out.

For any other repairs and services, contact **Technical Support**. Do not perform any repairs or services on the eStain® 2.0 Protein Staining Device by yourself to avoid damaging the device.

Protocols, continued

Replacing the eStain® 2.0 Graphite Electrode

During electric staining process, the eStain® 2.0 Graphite Electrode will absorb ions from anode pad while losing carbon composition, thereby changing the characteristics of the eStain® 2.0 Graphite Electrode and composition of the anode buffer.

For best staining results, after **100 uses** of electric staining, the worn eStain® 2.0 Graphite Electrode should be replaced by a new one.

Caution: Do **Not** replace the graphite electrode immediately after ending the staining program

since the graphite electrode is still hot after a run. Wait for 3 to 5 minutes before proceeding to the replacement protocol.

1. If the eStain® 2.0 Protein Staining Device works at staining 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”, replace the eStain® 2.0 Graphite Electrode as described below.



2. Switch **Off** the eStain® 2.0 Protein Staining Device. Open the lid of the device and take the worn eStain® 2.0 Graphite Electrode out of the device.



3. Remove the new eStain® 2.0 Graphite Electrode from the packing box.

Note: Wear gloves when handling the eStain® 2.0 Graphite Electrode.

Protocols, continued

Replacing the eStain® 2.0 Graphite Electrode, continued

4. Insert the contact rods of the new eStain® 2.0 Graphite Electrode into the holes in the anode tank. Make sure that the triangle symbols at the edge of the graphite electrode face the **Open** button.



5. Push the eStain® 2.0 Graphite Electrode down gently until you hear a click.



Protocols, continued

Replacing the eStain® 2.0

Graphite Electrode,
continued

6. Close the lid and switch **On** the eStain® 2.0 Protein Staining 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.



After successfully installing the eStain® 2.0 Graphite Electrode into the eStain® 2.0 Protein Staining Device, you are ready to use the device for next staining application.

Note: For best results, after every 40 times of electric staining, wash the eStain® 2.0 Graphite Electrode by soaking in distilled water for half an hour followed by drying with tissue paper.

Troubleshooting

Problem	Cause	Solution
The right status light doesn't flash during electric staining process.	Incomplete electric circuit due to improper assembly of the staining stack.	Ensure the staining stack is assembled correctly: Use the eStain® Anode Pad first followed by the gel and eStain® Cathode Pad.
The left and right status lights flash simultaneously.	Excessive current is flowing through the eStain® 2.0 Device.	Check if the staining stack is properly assembled and ensure full coverage of the gel.
The stained gel has very faint or nearly invisible protein bands with high blue background.	1. The staining time is not long enough. 2. The eStain® 2.0 Graphite Electrode has been used for more than 100 times.	1. Cover the eStain® Cathode Pad again and then perform electric staining for 1 or 2 more minutes. 2. Replace the worn eStain® 2.0 Graphite Electrode with a new one.
The stained gel has very faint or nearly invisible protein bands with clear background.	The staining time is too long.	Do Not stain the gel using eStain® 2.0 System again. Instead, stain the gel using traditional tri-step method.
The stained gel has blue spots.	The gel has not been destained sufficiently.	Invert the eStain® Cathode Pad, cover it back on top of the gel. Perform electric staining for 1 or 2 more minutes.
Blue spots observed at the bottom of the stained gel.	1. The spring is missing. 2. The eStain® 2.0 Graphite Electrode is installed in wrong direction.	1. Fix the spring on the contact rod located close to triangle symbols. 2. Follow the steps described in the protocol to install the eStain® 2.0 Graphite Electrode in correct direction.

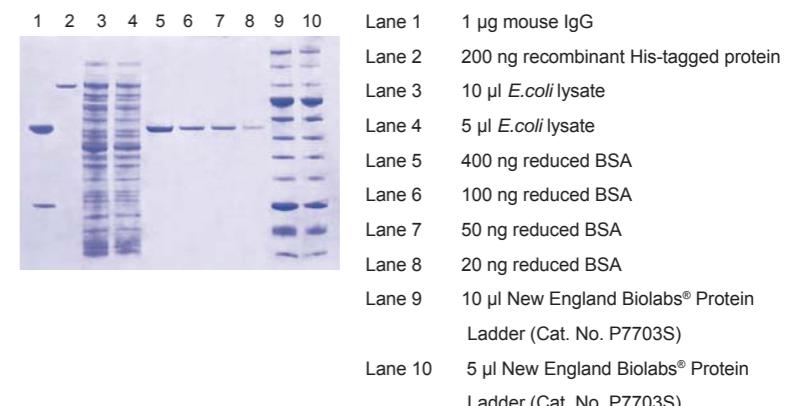
Troubleshooting, continued

Problem	Cause	Solution
Ring-like spots observed on the protein bands.	<ol style="list-style-type: none"> 1. Air bubbles trapped in the assembled staining stack. 2. Small gel pieces attached on the surface of the gel. 	<ol style="list-style-type: none"> 1. Use the small shovel supplied with eStain® 2.0 Device to press air bubbles out after staining stack assembly. 2. Make sure to remove all the gel pieces by washing the gel in distilled water prior to staining.
It is hard to close the lid or the lid can't be closed tightly	<ol style="list-style-type: none"> 1. The lid is not closed properly. 2. Extra Sponge Cushions are placed in the anode tank. 	<ol style="list-style-type: none"> 1. Follow the instruction on page 14 to properly close the lid. 2. Use 1 piece of Sponge Cushion when staining 1.5 mm gel; use 2 pieces of Sponge Cushion when staining 0.75 and 1mm gel.

Examples of Results

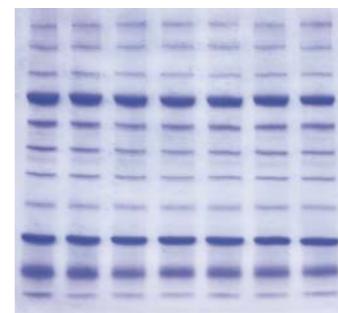
Tris-Glycine Gel Results

Samples were separated on a 12% Tris-Glycine gel and stained using eStain® 2.0 Protein Staining System with CBB R-250.



Bis-Tris Gel Results

7µl New England Biolabs® Protein Ladder (P7703S) were loaded in each lane and separated on 4-20% Express PAGE Gel (Bis-Tris gel). The gel was then stained using eStain® 2.0 Protein Staining System with CBB 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® 2.0 Protein Staining Device

GenScript warrants that eStain® 2.0 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 the 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.

Appendix



Appendix, continued



Appendix, continued

