INSTRUCTIONS

Pierce Midi Gel Power Staining Kit
Pierce Mini Gel Power Staining Kit

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Rev. A.0
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22839 22840

Number	Description
22839	**Pierce Midi Gel Power Staining Kit** **Note:** The kit contains sufficient reagents to stain 15 midi-sized gels at 30mL each. **Kit Contents:** **Midi Gel Pads,** 2 boxes of 15 each (8 sheets/pad) **Pierce Power Stain Solution,** 480mL **Destain Solution,** 480mL
22840	**Pierce Mini Gel Power Staining Kit** **Note:** The kit contains sufficient reagents to stain 30 mini-sized gels at 15mL each. **Kit Contents:** **Mini Gel Pads,** 2 boxes of 30 each (8 sheets/pad) **Pierce Power Stain Solution,** 480mL **Destain Solution,** 480mL

Storage: Upon receipt, store at room temperature. Product is shipped at room temperature.

Introduction

The Thermo Scientific™ Pierce™ Mini and Midi Gel Power Staining Kits are designed for rapid electrophoretic Coomassie staining of proteins in polyacrylamide gels when used with the Invitrogen™ Power Blotter System (Product No. PB0012) or the Thermo Scientific™ Pierce™ Power Stainer and the Thermo Scientific™ Pierce™ Power System.

Compared to traditional Coomassie staining techniques that can require protocols of 1 hour to overnight for staining and destaining, the Pierce Mini and Midi Gel Power Staining Kits provide rapid and efficient staining and destaining of SDS-PAGE protein gels in less than 20 minutes when used in conjunction with the Invitrogen Power Blotter System. This significant reduction in protein staining time is accomplished by using optimized ionic Power Stain Solution and Destain Solution to electrophoretically drive the negatively charged Coomassie R250 dye through the polyacrylamide gel towards the positively charged anode. The sulfonic acid groups in the Coomassie R250 dye bind ionically to the positive amine groups in the embedded protein, but not to the polyacrylamide gel matrix. This results in bright purple-stained protein bands with very low background. Pierce Gel Power Staining Kits have been validated to work with a wide variety of commonly used pre-cast and hand-cast SDS-PAGE gels (see Table 1).

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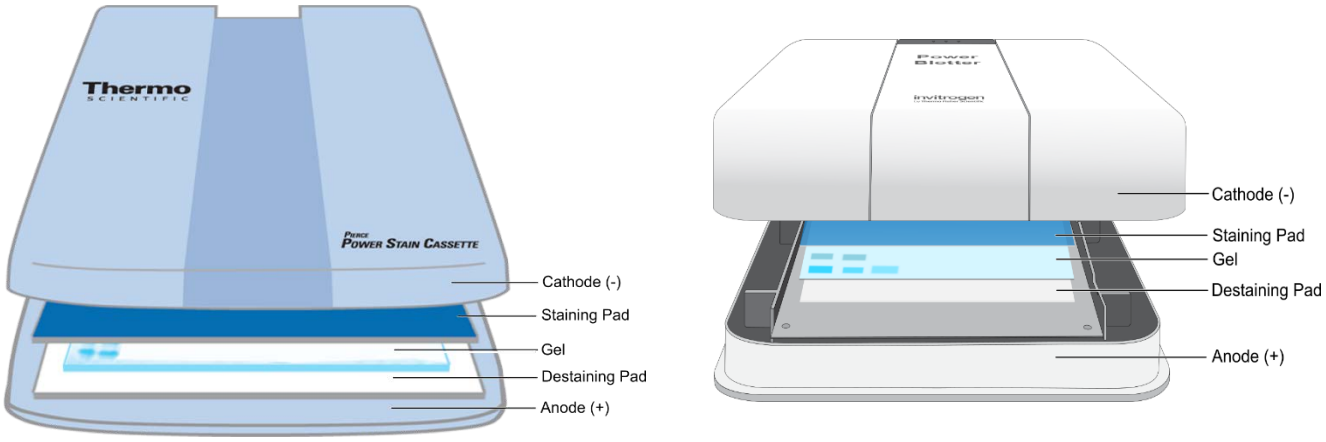

Figure 1. Thermo Scientific Pierce Power Stain Cassette (left) and Invitrogen Power Blotter Cassette (right).

Procedure

Important:

- These procedures were optimized for the following pre-cast gels: Novex™ Tris-Glycine gels, Bolt™ Bis-Tris Plus gels, NuPAGE™ Bis-Tris gels, Criterion™ Tris-HCl and Mini-PROTEAN™ TGX™ gels. Other gel types may require further optimization.
- It is recommended to designate the Invitrogen Power Blotter Cassette solely to staining or for blotting. Using the same cassette for both applications can result in contamination that could interfere in subsequent experiments.

A. Additional Materials Required

- Trays/containers to accommodate mini-sized (7 × 8.4cm) (e.g., Mini Gel Incubation Trays, Product No. 22843) and midi-sized gels (8 × 13.5cm) (e.g., Midi Gel Incubation Trays, Product No. 22841)
- Roller or other suitable device (e.g., Thermo Scientific™ Western Blot Roller, Product No. 84747)

B. Electrophoretic Staining of Polyacrylamide Gels

1. For each mini gel, use two mini gel pads (8 sheets/pad). For each midi gel, use two midi gel pads (8 sheets/pad).

   Note: Each gel pad (8 white sheets) is separated by one blue interleaf. Discard blue interleaf before placing gel pad in Power Stain or Destain Solution.

   Note: Gels simultaneously stained must have the same gel formulation.

2. Prepare destaining (bottom) pad: place one mini gel pad or one midi gel pad into tray and evenly add 15mL (mini gel) or 30mL (midi gel) of Destain Solution to the pad.
3. Prepare staining (top) pad: place one mini gel pad or one midi gel pad into tray and evenly add 15mL (mini gel) or 30mL (midi gel) of Power Stain Solution to the pad.
4. After electrophoresis, remove gel(s) from cassette and wash the mini gel(s) once for 5 minutes in deionized water on a shaker plate prior to staining. When staining two mini gels simultaneously or one midi gel, wash each gel twice for 5 minutes prior to staining.
5. Place the destaining pad on the surface of cassette bottom (anode) and roll gently to remove air bubbles. Use a clean roller or appropriate substitute (pipette).
6. Place the pre-washed gel on top of the destaining pad and roll gently to remove any air bubbles.
7. Place the staining pad on top of the gel and roll gently to remove any air bubbles.

8. Lock cassette top (cathode) into place and slide the assembled cassette into the Blotter Station.

A. If using the Thermo Scientific Pierce Power Stainer or Thermo Scientific Pierce G2 Fast Blotter:

a. Select **Begin Staining.**

b. Chose appropriate icon in the **Select Gel Number and Size** and **Choose Method** - refer to **Table 1** for gel type and pre-programmed method. Press **Start** button to begin staining.

Note: Staining 1/2 mini gel requires time optimization (approximately 2/3 of the time required to stain one mini gel of the same gel formulation).

B. If using the Invitrogen Power Blotter System with the Power Blotter Cassette (Product No. PB0002):

a. Press **Begin Blotting**.

b. Select **Custom Methods**.
Custom Methods can be used to create and run or create and saved a method on Power Blotter System for specific applications such as staining. Once created, the method will be displayed in Custom Methods and can be directly select. If a saved program is selected the ready screen will appear (skip to Step g).

c. Select the **Create Method** button and press **Select Constant (V or A)** to toggle the constant variable parameter from volts to amps. Leave voltage set at 25V as limit.

d. Refer to **Table 2** to determine the optimal amps and run time for the gel type and size to be stained. Press the **Up** or **Down** arrow to raise or lower the selected variable's value (amperage and/or time).

e. After the program is modified, press the **Done** button.

f. The Review Method screen is now displayed. Select **Run Without Saving** or **Save**.

- **Run Without Saving** will prompt the Ready screen. Proceed to Step g.
- If you wish to save the modified method, press **Save** and use the alphanumeric keypad to enter up to 15 characters to identify the new custom method. Press **Done** and the **Ready screen** will appear**.**

Note: The custom method will be saved in Custom Methods List which can be selected in Step b for future runs.

g. Press **Start** to begin the staining.

9. After staining is complete, rinse cassette under running water and allow to dry.

10. Store stained gel in a plastic protective sheet or in water for up to 4 hours.

Table 1. Recommended Thermo Scientific Pierce Power Stainer methods.

Gel Description	Gel Number and Size	
	1 mini gel	2 mini gels or 1 midi gel
Bolt™ Bis-Tris Mini Gels NuPAGE™ Bis-Tris Mini Gels Mini-PROTEAN™ TGX Gel	Type 1, 6:00	Type 1, 6:30
Novex™ Tris-Glycine Mini Gels Hand-cast Tris-Glycine Mini Gels	Type 2, 6:30	Type 2, 11:00
NuPAGE™ Bis-Tris Midi Gels Novex™ Tris-Glycine Midi Gels Criterion™ Tris-HCl Gels	—	Midi, 11:00

Table 2. Compatible gels and recommended gel staining settings for the Invitrogen Power Blotter.

Gel Brand/Type	Gel Chemistry	% Acrylamide	Size	Amps	Staining Time (min:sec)
Bolt	Bis-Tris	4-12%	Mini	1.3A	5 min
NuPAGE	Bis-Tris	4-12%	Mini	1.3A	5 min
Mini-PROTEAN	TGX	4-20%	Mini	1.3A	5 min
Novex	Tris-Glycine	4-20%	Mini	1.3A	6m 30s
Handcast	Tris-Glycine	10%	Mini	1.3A	6m 30s
Gel Brand/Type	**Gel Chemistry**	**% Acrylamide**	**Size**	**Amps**	**Staining Time**
NuPAGE	Bis-Tris	4-12%	Midi	1.3A	10 min
Novex	Tris-Glycine	4-20%	Midi	1.3A	10 min
Criterion	Tris-HCl	4-20%	Midi	1.3A	10 min
Bolt	Bis-Tris	4-12%	2-Mini	**2.5A**	5 min
NuPAGE	Bis-Tris	4-12%	2-Mini	**2.5A**	5 min
Mini-PROTEAN	TGX	4-20%	2-Mini	**2.5A**	5 min
Novex	Tris-Glycine	4-20%	2-Mini	1.3A	10 min
Handcast	Tris-Glycine	10%	2-Mini	1.3A	10 min

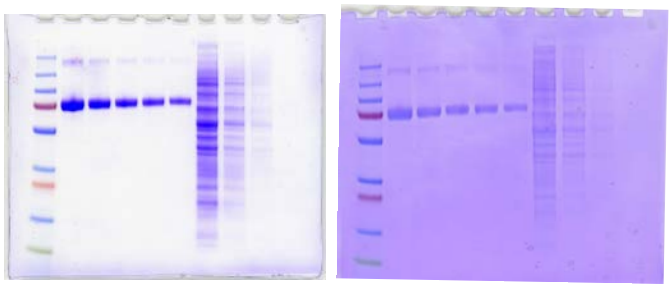

Figure 2. Rapid electrophoretic staining/destaining of Bolt 4-12% Bis-Tris gel vs. standard staining with Imperial™ Protein Stain. (Left image) Rapid staining: 1 × 5 minutes water wash and 5 minutes staining/destaining on Power Blotter using Pierce Mini Gel Power Staining Kit. (Right image) Standard Coomassie staining: 3 × 5 minutes water wash, 60 minutes staining using Imperial Protein Stain, and 60 minutes destaining in water (right gel).

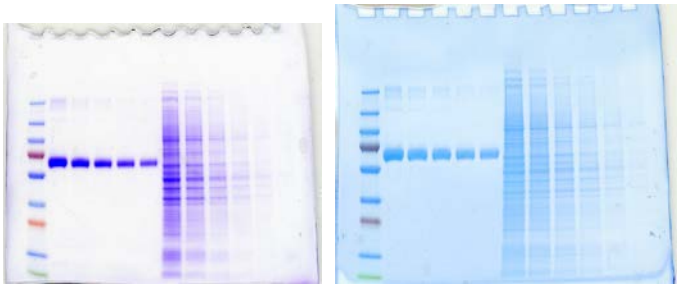

Figure 2. Rapid electrophoretic staining/destaining of Novex 4-20% Tris-Glycine gel vs. standard staining with GelCode™ Blue Safe Stain. (Left image) Rapid staining: 1 × 5 minutes water wash and 5 minutes staining/destaining on Power Blotter using Pierce Mini Gel Power Staining Kit. (Right image) Standard Coomassie staining: 3 × 5 minutes water wash, 60 minutes staining using Imperial Protein Stain, and 60 minutes destaining in water.

Troubleshooting

Problem	Possible Cause	Solution
Patchy background	Insufficient pre-wash time	Wash one mini-sized gel 1 × 5 minutes, two mini-sized gels 2 × 5 minutes and one midi-sized gel 2 × 5 minutes in water
	Uneven surface and/or air pockets were between gel and gel pads	When assembling the stack, use a roller or pipette to remove any air bubbles between the gel and the gel pads
	Insufficient staining/destaining time	Reassemble the staining/destaining stack and destain for an additional 1 to 1.5 minutes
		Place the gel in container with water to further destain
Inconsistent staining	Air pockets were trapped between gel and gel pads	When assembling sandwich, use a roller or pipette to remove any air bubbles between the gel and the gel pad
Low sensitivity	Staining time was too long	Reduce the staining time by increments of 30 seconds

Related Thermo Scientific Products

PB0012	**Invitrogen Power Blotter System**
PB0010	**Invitrogen Power Blotter Station**
PB0002	**Invitrogen Power Blotter Cassette**
22841	**Midi Gel Incubation Trays,** 10 each
22843	**Mini Gel Incubation Trays,** 10 each
84747	**Western Blot Roller**
26616	**PageRuler™ Prestained Protein Ladder, 10-170kDa,** 2 × 250µL
26619	**PageRuler Plus Prestained Protein Ladder,** 2 × 250µL
26634	**Spectra Multicolor Broad Range Protein Ladder,** 2 × 250µL
26614	**PageRuler Unstained Protein Ladder,** 2 × 250µL