

# Imperial™ Protein Stain

24615 24617

1737.1

| Number | Description  |
|--------|--|
| 24615  | <b>Imperial™ Protein Stain</b> , 1 L, sufficient reagent for up to 50 mini gels      |
| 24617  | <b>Imperial™ Protein Stain</b> , 3 × 1 L, sufficient reagent for up to 150 mini gels |

**Storage:** Upon receipt store product at room temperature.

## Introduction

Imperial™ Protein Stain is a coomassie R-250 dye-based reagent for protein staining in polyacrylamide gels. This sensitive ( $\leq 3$  ng) stain produces an intense color that photographs well. This reagent stains only protein and allows bands to be viewed directly in the gel during the staining process. The easy protocol is flexible to meet demanding time and sensitivity requirements and uses a simple water wash to yield a clear background.

## Procedure for Staining Gels

1. **SDS-PAGE:** After electrophoresis place gel in a clean tray and wash three times for 5 minutes with 50-100 ml of ultrapure water. Alternatively, wash gel in 200 ml of ultrapure water with gentle shaking for 15 minutes.

**Native PAGE:** Wash gel with 50-100 ml of ultrapure water for 5 minutes.

**Note:** Gels electrophoresed in MOPS or MES buffers may require an additional 5-minute wash (4 × 5 minutes total) for good sensitivity and reduced background.

2. Mix the Imperial™ Protein Stain immediately before use by gently inverting or tipping and swirling the bottle several times.

**Note:** Imperial™ Protein Stain contains additives that help slow formation of dye-dye aggregates, which occur in all coomassie dye-based protein staining reagents. If left undisturbed, the reagent may form visible aggregates that settle in the bottle; however, gentle mixing completely disperses these aggregates. To ensure that a homogeneous sample of the reagent is used, mix reagent before pouring or dispensing.

3. Add sufficient volume of stain to completely cover gel. Typically, 20-25 ml of stain is sufficient for an 8 × 10 cm gel. Place tray on a shaker. See Table 1 for staining time to achieve the desired level of sensitivity.

**Table 1.** Imperial Protein Stain detection sensitivity using various staining and wash times.

| <u>Sensitivity (ng)</u> | <u>Stain Time</u> | <u>Wash Time</u> |
|-------------------------|-------------------|------------------|
| $\leq 3$                | 2 hours           | overnight        |
| 3-6                     | 1 hour            | 1-2 hours        |
| 6-12                    | 5-10 minutes      | 3 × 5 minutes    |

4. Discard staining reagent and replace with 200 ml of ultrapure water to reduce background. Incubate on an orbital shaker. See Table 1 for wash time to achieve the desired level of sensitivity.

**Note:** Frequently replacing water and extending wash times will increase band intensity in contrast with the background. Placing a folded Kimwipe™ Tissue or paper towel in the container will enhance the destaining process by absorbing excess dye and will eliminate the need for replacing water. Remove tissue from the container when wash is complete. Overnight washing will not reduce band intensity.

## Microwave Procedure for Staining Gels

This procedure is fast, produces sharp bands and is sensitive to 6-12 ng. The procedure is optimized for standard 1 mm thick mini gels. Larger or thicker gels may require additional volumes of reagents or longer microwave and incubation times.

1. Place gel in a clean tray, add 100 ml of ultrapure water and microwave for 90 seconds.
2. Mix the Imperial™ Protein Stain immediately before use by gently inverting or tipping and swirling the bottle several times.
3. Discard water wash from gel and add 50 ml of stain or sufficient volume to completely cover the gel. Microwave gel for 90 seconds or until solution is about to boil. Do not let solution boil to evaporation.
4. Discard staining reagent and replace with 100 ml of ultrapure water. Microwave gel for 90 seconds. Place tray on an orbital shaker and incubate for 5 minutes.

**Note:** Placing an approximately 40 cm<sup>2</sup> folded Kimwipe™ Tissue (or paper towel) in the destaining container will enhance the destaining process by absorbing excess dye leaching from the gel.

## Procedure for Staining Nitrocellulose Membranes

1. Place nitrocellulose membrane containing transferred proteins in a tray and rinse for 1-2 minutes with ultrapure water.
2. Mix the Imperial™ Protein Stain immediately before use by gently inverting or tipping and swirling the bottle several times.
3. Add sufficient volume of stain to completely cover the membrane. Typically, 10 ml of stain is sufficient for 8 × 10 cm membrane. Incubate on an orbital shaker for 2-5 minutes.
4. Reduce background with a solution of 50% methanol/10% acetic acid for 4-10 minutes, replacing the solution 2-3 times.
5. Before drying the membrane for preservation, rinse it with 10% methanol to prevent wrinkling.

## Troubleshooting

| Problem             | Possible Cause      | Solution  |
|---------------------|---------------------|---|
| High background     | SDS interference    | Wash gel extensively before staining                              |
| No band development | Gel is > 1 mm thick | Destain background for 2-4 hours; alternatively, use thinner gels |

## Related Pierce Products

|             |   |
|-------------|---|
| 24612       | SilverSNAP® Stain Kit II  |
| 24597       | Color Silver Stain II Kit   |
| 24600       | SilverSNAP® Stain for Mass Spectrometry                               |
| 26681       | BlueRanger™ Prestained Protein Molecular Weight Marker Mix            |
| 24580       | MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes    |
| 28378       | BupH™ Tris-Glycine-SDS Running Buffer Packs                           |
| 25200-25244 | Precise™ Protein Gels, see catalog or web site for a complete listing |
| 28398       | BupH™ Tris-HEPES-SDS Running Buffer Packs                             |

U.S. patent pending on Imperial™ Protein Stain Technology

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The most current versions of product instructions are available at [piercenet.com](http://piercenet.com). For a faxed copy call 800-874-3723 or contact your local distributor.

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