INSTRUCTIONS

Thermo SCIENTIFIC

Imperial™ Protein Stain

Pub. No. MAN0011533
Rev. B.0
Pub Part No. 2161737.3

24615 24617

Number	Description
24615	**Imperial™ Protein Stain,** 1 L, sufficient reagent for up to 50 mini gels
24617	**Imperial™ Protein Stain,** 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 (≤ 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.

Sensitivity (ng)	Stain Time	Wash Time
≤ 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.

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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/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^2 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/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	No protein in sample	Load a known amount of purified protein as a control
	Insufficient amount of protein in sample	Load more total protein in gel
	SDS not completely removed from gel	Wash gel more extensively before staining

Additional Information

Visit the web site for additional information relating to this product including the following Tech Tip: Process stained polyacrylamide gels for mass spectrometry.

Related Products

24612	**Pierce™ Silver Stain Kit**
24600	**Pierce Silver Stain for Mass Spectrometry**
26619	**PageRuler Plus Prestained Protein Ladder, 10-250 kDa**
24580	**MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes**
NW04120BOX	**Bolt™ Bis-Tris Plus protein gels** (see thermofisher.com/proteingels for a complete listing)
B0002	**20X Bolt MES SDS Running Buffer**
B0001	**20X Bolt MOPS SDS Running Buffer**

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

Kimwipe™ is a trademark of Kimberly-Clark

For research use only. Not for use in diagnostic procedures.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Current versions of product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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