

Product Information

Phosphoprotein In-Gel Stain

Catalog Number	Packaging Size
P005A	500 mL

Storage upon receipt:

- 2-25°C
- Protect from light

Ex/Em: 550/580 nm

Product Description

Phosphoprotein In-Gel Stain is a high sensitive fluorescent stain designed for selectively detecting phosphoproteins in polyacrylamide gels. This Phosphoprotein In-Gel Stain containing a phos-tag group allows direct, in-gel detection of phosphate groups attached to tyrosine, serine, or threonine residues, without the need for antibodies or radioisotopes. The stain can be used with standard SDS-polyacrylamide gels or with 2-D gels. **Phosphoprotein In-Gel Stain** has the following advantages:

- **High sensitivity.** Detect as little as 1 ng protein.
- **Simple and fast staining.**
- **Compatibility with standard laboratory equipment.** Stained protein can be viewed using a visible-light transilluminator, or a 300 nm UV transilluminator.
- **Wide linear detection range.** At least three orders of magnitude.
- **Compatible with downstream analysis:** Compatible with MS and sequencing.
- **Stable:** Stable at room temperature for 1 year.

Sample Preparation

A delipidated and desalted sample is essential for adequate separation of proteins by electrophoresis and subsequent staining by Phosphoprotein In-Gel Stain.

- ❖ For a 150 µL sample (~150-300 µg of protein), add 600 µL of methanol and mix well by vortexing.
- ❖ Add 150 µL of chloroform and mix well by vortexing.
- ❖ Add 450 µL of ultrapure water and mix well by vortexing.
- ❖ Centrifuge at ~12,000 rpm for 5 min.
- ❖ Discard the upper phase, keeping the white precipitation disc that forms between the upper and lower phases.
- ❖ Add 450 µL of methanol and mix well by vortexing.
- ❖ Centrifuge at ~12,000 rpm for 5 minutes.
- ❖ Discard the supernatant and dry the pellet in a vacuum centrifuge for 10 minutes.
- ❖ Resuspend the pellet in standard 1X sample buffer for electrophoresis.

Staining Protocol

Note: The protocol is optimized for standard 1 mm thick, 8 cm x 8 cm SDS-PAGE minigels. Larger or thicker gels require additional volumes of reagents or longer incubation times.

1. **Run** gel as usual according to your standard protocol.
2. **Fix** gel with 100 mL of fix solution (50% methanol, 10% acetic acid), and agitate on an orbital shaker for 30 min. Repeat one more time with 100 mL fresh fix solution.
3. **Wash** the gel in 100 mL of ultrapure water with gentle agitation for 10 minutes. Repeat this step twice, for a total of three washes.
4. **Stain** the gel with enough **Phosphoprotein In-Gel Stain** (40~60 mL) to cover the gel, and agitate on an orbital shaker for 60-90 min.
5. **Destain** the gel with Phosphoprotein In-Gel Destain Solution (P005B) with gentle agitation for 30 minutes. Repeat this procedure two more times.
6. **Wash** the gel twice with ultrapure water for 5 minutes per wash. If the background is high or irregular, the gel may be left in the second wash for 20-30 minutes and re-imaged.
7. **Image** gel with a 300 nm UV transilluminator, blue-light transilluminator or a laser scanner.

Protocol Quick Guide

	Reagent	Protocol
Fix	50% methanol, 10% acetic acid	100 mL, 30 min
		100 mL, 30 min
Wash	Ultrapure water	100 mL, 10 min
		100 mL, 10 min
		100 mL, 10 min
Stain	Phosphoprotein In-Gel Stain	40-60 mL 60-90 minutes.
Destain	Phosphoprotein In-Gel Destain Solution	60 mL, 30 min
		60 mL, 30 min
		60 mL, 30 min
Wash	Ultrapure water	100 mL, 5 min
		100 mL, 5 min

Staining the Gel for Total Protein

After staining with Phosphoprotein In-Gel Stain, the gel can be stained with a total-protein stain.

1. **Image** the gel following staining with the first gel stain.
2. **Rinse** the gel with ultrapure water for 5 minutes. Repeat one more time.
3. **Incubate** gel with eLuminol Protein Gel Stain solution (40~60 mL, P003A&B). Microwave 45 seconds, and agitate on an orbital shaker for 15 min. Repeat microwave 45 seconds, and agitate on an orbital shaker for another 15 min.
4. **Wash** gel with 100 mL wash solution (10% methanol, 7% acetic acid) for 30 min.
5. **Image** gel with a 300 nm UV transilluminator, blue-light transilluminator or a laser scanner.

Related Products

Catalog No. Product
P005B Phosphoprotein In-Gel Destain Solution, 500mL