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Product Information

Phos-Tag™ Phosphoprotein 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

Phos-Tag™ Phosphoprotein Gel Stain is a high sensitive fluorescent stain designed for selectively detecting phosphoproteins in polyacrylamide gels. This stain contains a phos-tag™ group, which 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. **Phos-Tag™ Phosphoprotein Gel Stain** has the following advantages:

- **High sensitivity.** Detect as little as 1 ng phosphoprotein.
- **Simple and fast staining.**
- **Compatibility with standard laboratory equipment.**
- **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 Phos-Tag™ Phosphoprotein Gel Stain.

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

Staining Protocol

Note: The protocol is optimized for standard 1 mm thick, 8 cm × 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 **Phos-Tag™ Phosphoprotein Gel Stain** (40~60 mL) to cover the gel, and agitate on an orbital shaker for 60-90 min.
5. **Destain** the gel with **Phos-Tag™ Phosphoprotein 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 using recommended instruments and filter sets (see Table 1 for recommendations). A 300 nm UV transilluminator or a blue-light transilluminator can be also used for imaging. However, the sensitivity will be 10-fold lower.

Protocol Quick Reference

	Reagent	Protocol
Fix	50% metyhanol, 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	Phos-Tag™ Phosphoprotein Gel Stain	40-60 mL 60-90 minutes.
Destain	Phos-Tag™ Phosphoprotein 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 **Phos-Tag™ Phosphoprotein Gel Stain**, the gel can be stained with a total-protein stain.

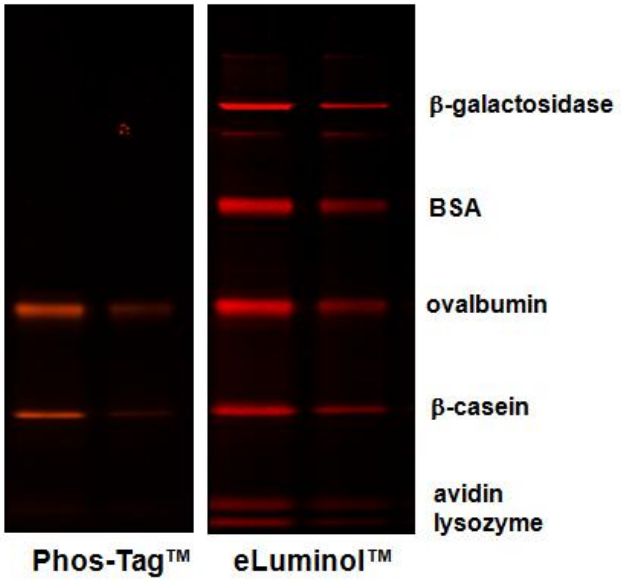
1. **Image** the gel following staining with the first gel stain.
2. **Rinse** the gel with ultrapure water for 5 minutes. Repeat one more time.
3. **Incubate** gel with eLuminol™ Protein Gel Stain solution (40~60 mL). Microwave 45 seconds, and agitate on an orbital shaker for 15 min. Repeat microwave 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
P003A	eLuminol™ Protein Gel Stain, 0.5 mL
P003B	eLuminol™ Protein Gel Stain, 1 mL

Table 1. Filters recommended for use with Phos-Tag™ Phosphoprotein Gel Stain

Instrument	Manufacturer	Excitation Source	Emission Filter
Typhoon Trio+, Trio, 9200, 9210, 9400, 9410	Amersham Biosciences	532 nm laser	560 nm longpass
FluorImager	Amersham Biosciences	514 nm laser	570 nm bandpass
Molecular Imager FX	Bio-Rad Laboratories, Inc	532 nm laser	555 nm longpass
FLA-3000G, FLA-5100	Fuji Photo Film Co, Ltd	532 nm laser	580 nm longpass
ProXPRESS	PerkinElmer LifeSciences, Inc	540/25 nm	590/30 nm



Protein gel stain results with Phos-Tag™ Phosphoprotein Gel Stain, followed by eLuminol™ Protein Gel Stain.