ABP Biosciences

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# Product Information

## Phospho-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

**Phospho-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. **Phospho-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 Phospho-Tag™ Phosphoprotein Gel Stain.

1. For a 150 μL sample (~150-300 μg of protein), add 600 μL of methanol and mix well by vortexing.
2. Add 150 μL of chloroform and mix well by vortexing.
3. Add 450 μL 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 μL 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 **Phospho-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 **Phospho-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% 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** | Phospho-Tag™ Phosphoprotein Gel Stain | 40-60 mL 60-90 minutes. |
| **Destain** | Phospho-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 **Phospho-Tag™ Phosphoprotein Gel Stain**, the gel can be stained with a total-protein stain.

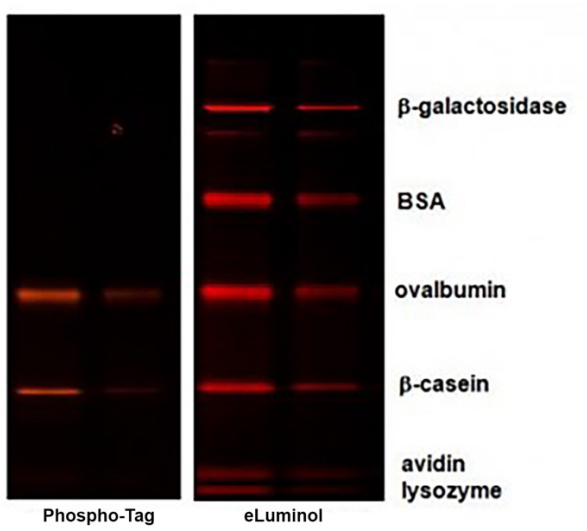
1. **Image** the gel following staining with the first gel stain.
2. **Rinse** the gel with ultrapure water for 5 minutes. Repeat one more time.
3. **Incubate** gel with eLuminol™ Protein Gel Stain solution (40~60 mL). Microwave 45 seconds, and agitate on an orbital shaker for 15 min. Repeat microwave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