LifeSct.com
Life Scientists' Service Center

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Phosphoprotein Gel Stain

Cat. #: C0118 Size: 500 ml

Introduction

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.

Package Information

Components	C0118
Phosphoprotein Gel Stain	500 ml

Ex/Em: 550/580 nm

Storage

Store at 2-25°C, Protect from light

Sample Preparation

A delipidated and desalted sample is essential for adequate separation of proteins by electrophoresis and subsequent staining by 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 1× 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 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 Phosphoprotein Destain Solution (Cat. #: C00119) 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	Phosphoprotein Gel Stain	40-60 mL 60-90 minutes.
Destain	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 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 SuperLumin™ Protein Gel Stain (Cat. #: C00117) 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.

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Table 1. Filters recommended for use with 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	532 nm laser	555 nm longpass
FLA-3000G, FLA-5100	Fuji Photo Film Co, Ltd	532 nm laser	580 nm longpass
ProXPRESS	PerkinElmer LifeSciences	540/25 nm	590/30 nm