

### Introduction

**Phosphoprotein Destain Solution** is used for phosphoprotein gel stain to reduce nonspecific binding. The destain solution is prepared with sodium acetate buffer and acetonitrile cosolvent. The destaining protocol is described in the gel staining protocol (C0118).

### Package Information

Components	C0119
Phosphoprotein Destain Solution	2×500 ml

### Storage

Store at 2-25°C

### 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 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
<b>Fix</b>	50% methanol, 10% acetic acid	100 mL, 30 min
		100 mL, 30 min
<b>Wash</b>	Ultrapure water	100 mL, 10 min
		100 mL, 10 min
		100 mL, 10 min
<b>Stain</b>	Phosphoprotein Gel Stain	40–60 mL 60–90 minutes.
		60 mL, 30 min
<b>Destain</b>	Phosphoprotein Destain Solution	60 mL, 30 min
		60 mL, 30 min
		60 mL, 30 min
<b>Wash</b>	Ultrapure water	100 mL, 5 min
		100 mL, 5 min