ABP Biosciences

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Product Information

SYPRO Ruby Protein Gel Stain

Catalog Number	Packaging Size
P002	1 L

Storage upon receipt:
- 2-25°C
- Protect from light

Ex/Em: 280, 450/610 nm

Product Description

SYPRO Ruby Protein Gel Stain is a ready-to-use, ultrasensitive, luminescent stain for the detection of proteins separated by polyacrylamide gel electrophoresis (PAGE). SYPRO Ruby stain is ideal for use in 1-D and 2-D PAGE. The sensitivity of SYPRO Ruby stain is as good as or better than the best silver stain.

Features:

- **High sensitivity:** 0.25-1 ng protein.
- **Wide linear detection range:** over three orders of magnitude.
- **Convenient:** ready-to-use 1X stain.
- **Simple protocol:** minimal hands-on time.
- **Compatibility with standard laboratory equipment.** Stained protein can be viewed using a 300 nm UV transilluminator, blue light transilluminator or a laser scanner.
- **Compatible with downstream analysis:** Compatible with MS and sequencing.
- **Stable:** Stable at room temperature for 1 year.

General Protocol Considerations

- Perform all fixation, staining, and washing steps with continuous, gentle agitation (e.g., on an orbital shaker at 50 rpm).
- Staining containers should be clean; we typically rinse the containers with ethanol before use. We recommend polypropylene or polycarbonate containers; these high-density plastics adsorb minimal amounts of the dye. For best results, use containers dedicated for SYPRO Ruby dye gel staining to minimize dye cross-contamination or other artifacts. Glass dishes are not recommended.
- The minimal recommended staining volume is approximately 10 times the volume of the gel in a container that is slightly larger than the dimensions of the gel. Use a sufficient volume so that staining solution flows over the surface of the gel. Using too little stain will result in reduced sensitivity.
- For convenience, gels may be left in fix solution overnight or longer.
- For convenience, gels may be left in SYPRO Ruby stain indefinitely without overstaining, although speckling artifacts tend to increase over time.
- As with any fluorescent stain, cover the gel container during staining and subsequent wash steps to exclude light.

Protocol Quick Reference

	Reagent	Basic Protocol	Rapid Protocol
Fix	50% methanol, 7% acetic acid	100 mL, 30 min	100 mL, 15 min
		100 mL, 30 min	100 mL, 15 min
Stain	SYPRO Ruby stain	60 mL, overnight	60 mL Microwave 30s Agitate 30s Microwave 30s Agitate 5 min Microwave 30s Agitate 23 min
Wash	10% methanol, 7% acetic acid	100 mL, 30 min	100 mL, 30 min
Hands-on time		10 min	15 min
Total time		~16 h	90 min

Preparation of Solutions

The basic protocol and the rapid protocol are both optimized for standard 1 mm thick, 8 cm × 8 cm SDS-PAGE minigels. The volumes of fix, staining, and wash solutions are easily optimized for larger or thicker gels. Use 20 times the volume of the gel for each fixation and wash step, and 10 times the volume of the gel of SYPRO Ruby stain.

Fix Solution

Prepare a fix solution of 50% methanol, 7% acetic acid. Prepare 200 mL fix solution per minigel or 1.6 L fix solution for each large format gel (i.e., 20 cm × 20 cm × 1 mm).

Note: For IEF gels, prepare a fix solution of 40% methanol, 10% trichloroacetic acid. Prepare 100 mL of this solution, or an amount equal to 20 times the volume of the gel.

Wash Solution

Prepare a wash solution of 10% methanol, 7% acetic acid. Prepare 100 mL wash solution per minigel or 800 mL wash solution for each large format gel (i.e., 20 cm × 20 cm × 1 mm).

Basic Protocol

Notes on the Basic Protocol

- The basic protocol is optimized for standard 1 mm thick, 8 cm × 8 cm SDS-PAGE minigels, such as Invitrogen NuPAGE Novex Bis-Tris and Novex Tris-glycine gels, and Novex Tricine gels.
- The basic protocol can also be used for nondenaturing gels, and IEF gels.
- Larger or thicker gels require additional volumes of reagents and/or longer incubation times. The basic staining protocol is easily optimized. Use 20 times the volume of the gel for fix and wash solutions, and 10 times the volume of the gel for the staining solution.

1.1 Fix. After electrophoresis, place the gel into a clean container with 100 mL of fix solution and agitate on an orbital shaker for 30 minutes. Repeat once more with fresh fix solution. Pour off the used fix solution.

Note: For IEF gels, place the gel into a clean container with 100 mL of IEF fix solution and agitate on an orbital shaker for 3 hours. After fixing, perform 3 washes in ultrapure water for 10 minutes each, before proceeding to the staining step.

1.2 Stain. Add 60 mL of SYPRO Ruby gel stain. Agitate on an orbital shaker overnight.

1.3 Wash. Transfer the gel to a clean container and wash in 100 mL of wash solution for 30 minutes. The transfer step helps minimize background staining irregularities and stain speckles on the gel. Before imaging rinse the gel in ultrapure water a minmum of two times for 5 minutes to prevent possible corrosive damage to the imager.

Rapid Protocol

The rapid stain protocol requires only 90 minutes. While the maximum fluorescence signal strength is lower than for the overnight protocol, the rapid protocol results in lower background and fewer speckling artifacts, allowing sensitivities as low as 0.25 ng in 1-D gels. Similar sensitivity is obtained in 2-D gels.

Notes on the Rapid Protocol

- The rapid protocol is optimized for standard 1 mm thick, 8 cm × 8 cm SDS-PAGE minigels, such as Invitrogen NuPAGE Novex Bis-Tris and Novex Tris-glycine gels, and Novex Tricine gels. The protocol is easily optimized for larger gels. Use 20 times the volume of the gel for fix and wash solutions, and 10 times the volume of the gel for the staining solution. Microwave times should be optimized for large format gels. Microwave the stain on full power for 30-45 seconds per round, until the stain reaches 80–85°C.
- Although SYPRO Ruby stain solution is not flammable, use caution when microwaving SYPRO Ruby stain as the solution becomes very hot. Wear eye protection, gloves and lab coat during all procedures. Placing a lid loosely over the staining container will minimize fumes. Do not heat the fixative solution or other methanolic solutions in the microwave.

2.1 Fix. After electrophoresis, place gel into a microwavable container with 100 mL of fix solution and agitate on an orbital shaker for 15 minutes. Repeat once more with fresh fix solution. Pour off the used fix solution.

2.2 Stain. Add 60 mL of SYPRO Ruby gel stain. Microwave 30 seconds, agitate 30 seconds to distribute heat evenly, microwave another 30 seconds to 80-85°C, and agitate on an orbital shaker for 5 minutes. Reheat the gel by microwaving a third time for 30 seconds and then agitate on an orbital shaker for 23 minutes for a total stain time of 30 minutes. An acceptable alternative to the microwave procedure is to incubate the gel at 80°C in a shaking water bath for 30 minutes.

2.3 Wash. Transfer the gel to a clean container and wash in 100 mL of wash solution for 30 minutes. The transfer step is necessary to avoid heating the destain solution, which may reduce stain sensitivity, and also helps minimize background staining irregularities and stain speckles on the gel. Before imaging, rinse the gel in ultrapure water a minimum of two times for 5 minutes to prevent possible corrosive damage to the imager.

Using SYPRO Ruby Stain As a Post-Stain

SYPRO Ruby stain can be used subsequent to staining with other gel stains such as Pro-Q Diamond phosphoprotein gel stain, Pro-Q Emerald 300 glycoprotein gel stain, Pro-Q Sapphire oligohistidine-tag gel stains. SYPRO Ruby stain should be used last, because its bright fluorescent signal tends to dominate the signal from other stains. SYPRO Ruby stain does not work well as a post-stain for colorimetric stains such as Coomassie and silver stains.

3.1 Image the gel following staining with the first gel stain.
3.2 Rinse the gel with ultrapure water for 5 minutes. Repeat one more time.
3.3 Incubate the gel directly in SYPRO Ruby gel stain solution. There is no need to repeat the fixation step.
3.4 Continue with the basic or rapid stain protocol and imaging method just as when using SYPRO Ruby stain alone.

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Viewing and Photographing the Gel

SYPRO Ruby protein gel stain has two excitation maxima, one at ~280 nm and one at ~450 nm, and has an emission maximum near 610 nm. Proteins stained with the dye can be visualized using a 300 nm UV transilluminator, a blue-light transilluminator, or a laser scanner. The stain has exceptional photostability allowing long exposure times for maximum sensitivity.

UV or Blue-Light Transilluminator

Proteins stained with SYPRO Ruby protein gel stain are readily visualized using a UV or blue-light source. The use of a photographic camera or CCD camera and the appropriate filters is essential to obtain the greatest sensitivity. The instrument's integrating capability can make bands visible that cannot be detected by eye.

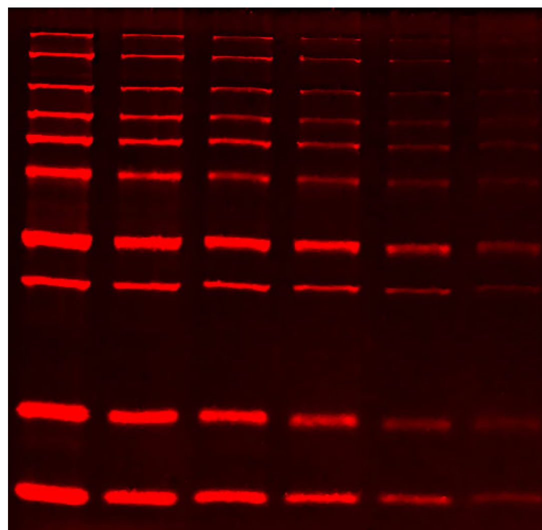
We use a 300 nm transilluminator with six 15 W bulbs. Excitation with different UV wavelengths may not give the same sensitivity.

Using a Polaroid camera and Polaroid 667 black-and-white print film, the highest sensitivity is achieved with a 490 nm longpass filter, such as the SYPRO photographic filter (S6656). We typically photograph minigels using an f-stop of 4.5 for 1 second.

Using a CCD camera, images are best obtained by digitizing at about 1024 × 1024 pixels resolution with 12- or 16-bit grayscale levels per pixel. A CCD camera–based image analysis system can gather quantitative information that will allow comparison of fluorescence intensities between different bands or spots.

Laser-Scanning Instruments

Gels stained with the SYPRO Ruby protein gel stain are readily visualized using imaging systems equipped with lasers that emit at 450, 473, 488, or 532 nm.



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