

SYPRO[®] Ruby Protein Gel Stain

S-12000 SYPRO[®] Ruby protein gel stain, 1 L

S-12001 SYPRO[®] Ruby protein gel stain, 200 mL

S-21900 SYPRO[®] Ruby protein gel stain, 5 L

Quick Facts

Storage upon receipt:

- Room temperature
- Protect from light

Ex/Em: 280, 450/610 nm

Introduction

Molecular Probes' SYPRO[®] Ruby protein gel stain is a ready-to-use, ultrasensitive, luminescent stain for the detection of proteins separated by polyacrylamide gel electrophoresis (PAGE). This stain, designed especially for use in 2-D PAGE, has proven to be our most sensitive protein gel stain for standard 1-D SDS-PAGE and an excellent stain for isoelectric focusing (IEF) gels, as well. SYPRO Ruby protein gel stain attains comparable sensitivity to that of the best silver-staining techniques.¹⁻³ However, *unlike* silver staining, the SYPRO Ruby gel stain:

- Uses a simple staining protocol, with no possibility of overstaining
- Delivers a linear quantitation range of over three orders of magnitude¹
- Shows less protein-to-protein variability
- Stains glycoproteins, lipoproteins, calcium binding proteins, fibrillar proteins and other difficult-to-stain proteins¹
- Will not stain extraneous nucleic acids¹
- Does not interfere with subsequent analysis of proteins by Edman-based sequencing or mass spectrometry^{1,2}

SYPRO Ruby protein gel stain is easy to use and is ideal for high-throughput gel staining. The SYPRO Ruby stain can be used with many types of gels, including 2-D gels, Tris-glycine SDS gels, Tris-tricine pre-cast SDS gels and nondenaturing gels. SYPRO Ruby stain is also compatible with gels adhering to plastic backings.

Materials

Contents

SYPRO Ruby protein gel stain is supplied ready to use in three sizes:

- **SYPRO Ruby protein gel stain, 200 mL (S-12001)** provides sufficient material to stain ~4 minigels
- **SYPRO Ruby protein gel stain, 1 L (S-12000)** provides sufficient material to stain ~20 minigels or 2–3 large-format gels
- **SYPRO Ruby protein gel stain, 5 L (S-21900)** provides sufficient material to stain ~100 minigels or 10–15 large-format gels

Storage

SYPRO Ruby protein gel stain is stable for at least 6 months when stored at room temperature, protected from light. For convenient storage and dispensing, the 5 L unit size is packaged in a cubical box with a spigot. Once opened, the box can be stored on its side with the top flap closed to protect the stain from light.

Disposal

No data are available on the toxicity of SYPRO Ruby protein gel stain. The stain comprises an organic component and a heavy metal component (ruthenium). Solutions of SYPRO Ruby gel stain, acidified by addition of a small amount of glacial acetic acid, should be poured through activated charcoal or other compatible combustible material and then burned in a chemical incinerator equipped with suitable afterburner/scrubber system to destroy the dye. All federal, state and local environmental regulations should be observed when disposing the dyes.

Staining Protocol

Perform SYPRO Ruby gel staining with continuous, gentle agitation (e.g. on an orbital shaker at 50 rpm). We have found that polypropylene dishes, such as Rubbermaid[®] Servin' Savers, are the optimal containers for staining because the high-density plastic adsorbs only a minimal amount of the dye. Containers should be clean; we typically rinse the containers with ethanol before use. For small gels, circular staining dishes provide the

best fluid dynamics on orbital shakers, resulting in less dye aggregation and better staining. For large-format 2-D gels, polyvinyl chloride photographic staining trays, such as Photoquip Cesco-Lite 8 inch × 10 inch photographic trays also work well. Glass dishes are not recommended.

1.1 Fix the gel. For 2-D gels, fix for 30 minutes in any of the following solutions:

- 10% methanol, 7% acetic acid
- 25% ethanol, 12.5% trichloroacetic acid
- 10% ethanol, 7% acetic acid
- 50% ethanol, 3% acetic acid
- 40% ethanol, 10% acetic acid

It is also possible to use serial combinations of these fixatives. Note that the combination of ethanol and acetic acid can result in the formation of ethyl acetate, which is not only toxic, but may interfere with identification of proteins by mass spectrometry.

For IEF gels, fix for 3 hours in 40% methanol, 10% trichloroacetic acid and then perform 3 washes in dH₂O, for 10 minutes each, before proceeding to the staining step.

A fixation step is not required for 1-D SDS polyacrylamide gels.

1.2 Incubate the gel in the undiluted stain. For maximum sensitivity in 1-D or 2-D gels, incubate the gel for at least 3 hours. For IEF gels, incubate the gel overnight.

- The minimal staining volumes for typical gel sizes are:

50 mL, for 8 cm × 10 cm × 0.75 mm gels
330 mL, for 16 cm × 20 cm × 1 mm gels
500 mL, for 20 cm × 20 cm × 1 mm gels

or ~10 times the volume of the gel for other gel sizes. Using too little stain will lower the sensitivity.

- For convenience, gels may be left in the dye solution overnight or longer without overstaining.
- Do not dilute the stain, as diluted stain will result in decreased sensitivity.
- Do not reuse the staining solution as this will result in a significant loss of sensitivity.

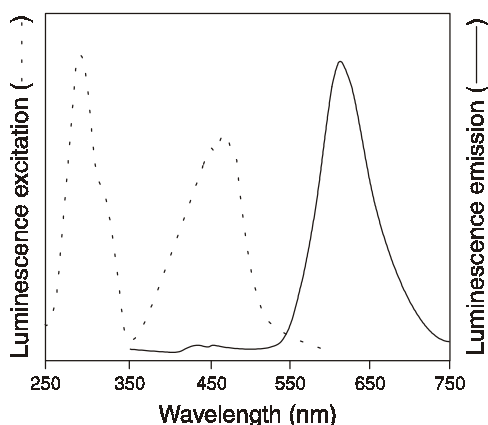


Figure 1. Excitation (dashed line) and emission (solid line) spectra for SYPRO Ruby protein gel stain.

1.3 Wash the gel. To reduce background fluorescence and increase sensitivity, wash the gel in 10% methanol (or ethanol), 7% acetic acid for 30 minutes. For IEF gels, perform this wash step three times. To reduce organic waste, stained gels may alternatively be washed in dH₂O, although this method does not reduce background fluorescence to the same extent. The gel may be monitored periodically using UV epi-illumination to determine the level of background fluorescence.

1.4 Optional. Dry the stained gel for permanent storage. Incubate the gel in a solution of 2% glycerol for 30 minutes. Dry the stained gel using a gel dryer. Note that proteins present at very low levels may no longer be detectable after gel drying.

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 (Figure 1). 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.

- It is important to clean the surface of the transilluminator after each use with deionized water and a soft cloth (like cheesecloth). Otherwise, fluorescent dyes, such as SYPRO stains, SYBR[®] stains and ethidium bromide, will accumulate on the glass surface and cause a high background fluorescence.
- We use a 300 nm transilluminator with six 15-watt bulbs. Excitation with different light sources 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 protein gel stain photographic filter (S-6656), available from Molecular Probes. We typically photograph mini-gels 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 gray scale levels per pixel. Please contact your camera manufacturer for recommendations on filter sets to use. A CCD camera-based image analysis system can gather quantitative information that will allow comparison of fluorescence intensities between different bands or spots. Using such a system, we have found that the SYPRO Ruby gel stain has a linear dynamic range over three orders of magnitude.
- The polyester backing on some premade gels is highly fluorescent. For maximum sensitivity using a UV transilluminator, the gel should be placed polyacrylamide side down and an emission filter, such as the SYPRO protein gel stain photographic filter (S-6656), used to screen out the blue fluorescence of the plastic. The use of a blue-light transilluminator or laser scanner will reduce the amount of fluorescence

from the plastic backing so that the gel may be placed polyester side down.

Laser-scanning instruments. Gels stained with the SYPRO Ruby protein gel stain can be visualized using imaging systems equipped with lasers that emit at 450, 473, 488 or 532 nm. For information on appropriate filter sets to use with common laser scanners, see our reference table at www.probes.com/handbook/tables/0388.html.

Subsequent Microanalysis of Proteins

SYPRO Ruby protein gel stain does not interact covalently with proteins. Edman-based sequencing or mass spectrometry data can be obtained immediately after staining.

Mass Spectrometry

The following protocol for preparing stained proteins for mass spectrometry is abstracted from *Electrophoresis* 19, 1920 (1998).

2.1 After staining gels with SYPRO Ruby Protein Gel Stain, excise protein bands or spots from the gel.

2.2 Wash the gel slices twice with 25 mM ammonium bicarbonate in 50% acetonitrile and once with 100% acetonitrile.

2.3 Dry the gel slices using a SpeedVac® concentrator.

2.4 Add approximately 20 µL of 20 µg/ml sequencing grade modified trypsin to the dried residue and incubate the sample overnight at 37°C.

2.5 Extract the peptides from the gel slices with 50 µL of 50% acetonitrile, 5% trifluoroacetic acid.

2.6 Dry the extract under vacuum.

2.7 Resuspend the peptides in 2 µL of 50% acetonitrile, 5% trifluoroacetic acid.

2.8 Spot 0.5 µL onto the plate of a MALDI-TOF mass spectrometer.

2.9 Apply 0.5 µL of 10 mg/ml α-cyanocinnamic acid to the spot and air dry at room temperature prior to acquiring mass spectra.

2.10 Perform mass spectrometry.

References

1. *Electrophoresis* 21, 2509 (2000); 2. *Electrophoresis* 21, 3673 (2000); 3. *Electrophoresis* 21, 1037 (2000).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
S-6656	SYPRO® protein gel stain photographic filter	each
S-12000	SYPRO® Ruby protein gel stain	1 L
S-12001	SYPRO® Ruby protein gel stain	200 mL
S-21900	SYPRO® Ruby protein gel stain *bulk packaging*	5 L

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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