SYPRO® Ruby Protein Blot Stain (S-11791)

Quick Facts

Storage upon receipt:
- Room temperature
- Protect from light

Abs/Em: 280, 450/618 nm

Introduction

The fluorescent SYPRO® Ruby protein blot stain provides a rapid, simple and highly sensitive method for detecting proteins on nitrocellulose or polyvinylidene difluoride (PVDF) membranes (blots). Staining total protein before specific protein detection techniques provides an assessment of protein transfer efficiency, and makes it possible to detect contaminating proteins in the sample and to compare the sample with molecular weight standards. For blots of 2-D gels, total-protein staining makes it easier to localize a protein to a particular spot in the complex protein pattern. The superior properties of SYPRO Ruby protein blot stain, compared to conventional protein blot stains, make it possible to routinely obtain this valuable information without resorting to running duplicate blots. The bright, orange-red–fluorescent stain can be easily visualized using UV illumination or a laser scanner. SYPRO Ruby protein blot stain is:

- **Easy to Use.** The staining procedure is simple to perform and complete within an hour.
- **Highly Sensitive.** The stain has a sensitivity limit of ~0.25–1 protein/mm^2 (2–8 ng/band), rivaling that of colloidal gold stains, and making it about 60 times more sensitive than reversible stains like Ponceau S and 20–30 times more sensitive than Amido Black or Coomassie Brilliant Blue stains.[1]
- **Selective for Proteins.** SYPRO Ruby stain does not detect contaminating nucleic acids.[1]
- **Compatible with Immunodetection Techniques.** SYPRO Ruby stain is fully compatible with colorimetric, fluorogenic and chemiluminescent detection techniques.[1,2] In contrast, Amido Black, Coomassie Brilliant Blue and colloidal gold stains often block epitopes required for subsequent immunodetection and the dark color of the stains makes it difficult to visualize colorimetric or fluorogenic immunodetection reagents.[3]
- **Compatible with Edman Sequencing and Mass Spectrometry.** The stain does not covalently modify proteins, ensuring accurate identification of proteins by Edman sequencing or mass spectrometry.[1]

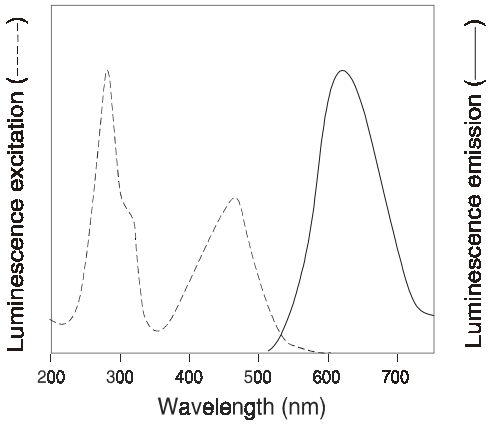

Figure 1. *Luminescence excitation and emission spectra of SYPRO Ruby blot stain.*

Materials

Contents

SYPRO Ruby protein blot stain is provided in a unit size of 200 mL. The 200 mL volume is sufficient for staining 10–40 minigel electroblots or four large-format electroblots (20 × 20 cm). SYPRO Ruby protein blot stain may be re-used up to four times with little loss in sensitivity.

Storage and Handling

The reagent is stable for at least 6 months to one year when stored at room temperature, protected from light.

Disposal

No data are available on the toxicity of SYPRO Ruby blot stain. The stain comprises an organic component and a heavy metal component (ruthenium). Solutions of SYPRO Ruby blot stain, acidified by the 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 of the dyes.

Staining Protocols

Staining Proteins After Electroblotting to Nitrocellulose Membranes

1.1 Perform all washing and staining steps with continuous, gentle agitation (e.g. on an orbital shaker at 50 rpm). After electroblotting proteins to a sheet of nitrocellulose membrane, completely immerse the membrane in 7% acetic acid, 10%

methanol and incubate at room temperature for 15 minutes in a small, polypropylene staining dish.

1.2 Incubate the membrane in four changes of deionized water for 5 minutes each.

1.3 Completely immerse the membrane in SYPRO Ruby blot stain reagent for 15 minutes.

1.4 Wash the membrane 4–6 times for 1 minute in deionized water. This wash serves to remove excess dye from the membrane. Membranes stained with SYPRO Ruby blot stain should be monitored using UV epi-illumination periodically to determine if background luminescence has been washed away.

1.5 Blots treated with the SYPRO Ruby blot stain are best preserved by allowing membranes to air dry. After staining, wet membranes should not be touched, since residue found on latex gloves may destroy the staining pattern. Use forceps to handle wet blots. Once dry, membranes can be handled freely.

Staining Proteins After Electroblotting to PVDF Membranes

2.1 Perform all washing and staining steps by floating the membrane face down on the solution and gently agitating (e.g. on an orbital shaker at 50 rpm). After electroblotting proteins to a sheet of PVDF membrane, allow the membrane to dry completely.

2.2 Float the membrane face down in 7% acetic acid, 10% methanol and incubate for 15 minutes.

2.3 Float the membrane for 5 minutes each in four changes of deionized water.

2.4 Float the membrane in SYPRO Ruby Blot stain reagent for 15 minutes.

2.5 Wash the membrane 2–3 times for 1 minute in deionized water. This wash serves to remove excess dye from the membrane.

2.6 Blots treated with the SYPRO Ruby blot stain are best preserved by allowing the membranes to air dry. After staining, wet membranes should not be touched, since residue found on latex gloves may destroy the staining pattern. Use forceps to handle wet blots. Once dry, the membranes can be handled freely.

Viewing and Photographing the Blot

SYPRO Ruby protein blot stain has two excitation maxima, one at ~280 nm and one at ~450 nm, and has an emission maximum near 618 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 epi- or transilluminator. Proteins stained with SYPRO Ruby protein blot stain are readily visualized using UV illumination. The front face of membranes can be illuminated using a hand held, UV-B (~300 nm) light source. Alternatively, a UV light box can be placed on its side to illuminate the blots or a top illuminating system such as the BioRad Fluor-S™ imager can be used to visualize the stain. Satisfactory results are obtained from direct transillumination through the blotting membrane as well. In either case, *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.
- 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 blots 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.

Laser-scanning instruments. Blots stained with the SYPRO Ruby protein blot 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 in Chapter 9.3 of our online *Handbook of Fluorescent Probes and Research Products* (www.probes.com/handbook).

Subsequent Microanalysis

Proteins may be analyzed by immunostaining, glycoprotein staining or mass spectrometry immediately after staining. It is important to photograph or otherwise document the SYPRO Ruby stain before immunostaining, as over 90% of the stain is washed off the blot during the blocking step. For Edman-based microsequencing, use a PVDF membrane, stain with SYPRO Ruby protein blot stain as described above and then partially destain the blot using the following procedure:

3.1 Wash the blot by placing it face down on a solution of 150 mM Tris, pH 8.8/20% methanol for 10 minutes, with gentle agitation.

3.2 Rinse the blot four times for 1 minute each in dH_2O.

3.3 Air dry the blot.

References

1. Anal Biochem 276, 129 (1999); **2.** Electrophoresis 21, 2196 (2000); **3.** Electrophoresis 19, 752 (1997).

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-11791	SYPRO® Ruby protein blot stain *10-40 blots*	200 mL

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.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

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