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SYPRO® Ruby Protein Blot Stain

Introduction

SYPRO® Ruby Protein Blot Stain is a highly sensitive fluorescent stain for the rapid detection of proteins following transfer to common blotting membranes such as nitrocellulose and polyvinylidene difluoride (PVDF). Using SYPRO® Ruby Blot Stain prior to performing specific protein detection techniques provides an assessment of protein transfer efficiency, allows for the detection of contaminating proteins, and enables the comparison of proteins of interest to molecular weight standards. SYPRO® Ruby Protein Blot Stain provides the following:

- Simple staining protocol, completed within 1 hour
- Specific for proteins. Will not detect contaminating nucleic acids
- Fully compatible with fluorogenic, chemiluminescent, and colorimetric detection techniques
- No possibility of overstaining
- Does not interfere with mass spectrometry or Edman-based sequencing
- High sensitivity

Contents

SYPRO® Ruby Protein Blot Stain is available in a 200 ml bottle and supplied ready-to-use. This is sufficient volume for staining 10-40 minigel electroblots or four large format electroblots (~20 x 20 cm). This stain can be reused up to four times without significant loss in sensitivity.

Storage and Handling

SYPRO® Ruby Protein Blot Stain should be stored at room temperature and protected from light. Under these conditions, the stain is stable for 2 years.

Disposal

Stain solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. All federal, state, and local environmental regulations should be observed when disposing of the stain.

Tips for Staining

- **Provide continuous, gentle agitation for all washing and staining steps** – An orbital shaker step at ~ 50 rpm will produce the proper level of agitation.
- **Stain in polypropylene dishes** – The high-density plastic of these containers adsorb a minimal amount of dye and increase staining efficiency. Rubbermaid® Servin' Savers® are an example of an appropriate container.
- **For large format gels, stain in polyvinyl chloride photographic staining trays** – 8 in x 10 in photographic trays work well.
- **Containers should be clean** – Rinse containers with ethanol prior to use.
- **Use circular staining dishes for staining small membranes** – The use of these containers results in less dye aggregation and better staining.
- **Glass dishes are <u>not</u> recommended.**

Staining Proteins blotted onto Nitrocellulose Membranes

1. Run gels and electroblot according to standard protocols.
2. Fix the membrane in a solution of 7% acetic acid/10% methanol at room temperature for 15 minutes. Ensure that the membrane is fully immersed during fixation.
3. Wash membranes in four consecutive changes of deionized water for 5 minutes each.
4. Stain using SYPRO® Ruby Protein Blot Stain for 15 minutes. Ensure that the membrane is fully immersed in the staining solution.
5. Wash the membrane in five consecutive changes of deionized water for 1 minute each. Greater or fewer washes can be done to maximize sensitivity and minimize background fluorescence.
6. Membranes can be dried for permanent storage. After staining, **do not** touch the membranes as this can destroy the staining pattern. Use forceps to handle the wet blots. Dry membranes can be handled freely.

Staining Proteins Blotted onto PVDF Membranes

1. Run gels and electroblot according to standard protocols. After electoblotting proteins onto a PVDF membrane, allow the membrane to dry completely prior to proceeding.
2. Fix the membrane in a solution of 7% acetic acid/10% methanol at room temperature for 15 minutes. Float the membrane face down to ensure fixation.
3. Float the membrane in four changes of deionized water for five minutes each to wash.
4. Float the membrane in SYPRO® Ruby Protein Blot Stain for 15 minutes.
5. Wash the membrane 3 times in deionized water for 1 minute each to remove excess dye and reduce background fluorescence.
6. Membranes can be dried for permanent storage. After staining, **do not** touch the membranes as this can destroy the staining pattern. Use forceps to handle the wet blots. Dry membranes can be handled freely.

Photographing Membranes Stained with SYPRO® Ruby Protein Blot Stain

SYPRO® Ruby Protein Blot Stain has two excitation maxima (at 280 nm and 450 nm), and one emission maximum (at 618 nm). The stained gels can be visualized using a 300 nm UV transilluminator, a blue light transilluminator, or a laser scanner. SYPRO® Ruby Protein Blot Stain remains photostabile over long exposure times.

- Clean the surface of the transilluminator prior to and after each use using deionized water and a soft cloth. Fluorescent dyes can accumulate on the glass surface and cause unacceptable background fluorescence.
- The front face of membranes can be illuminated by either using a handheld UV-B (~300 nm) light source, or by placing a UV light box on its side to illuminate the blots. Alternatively, a top illuminating system such as the Bio-Rad Fluor-S™ can be used.
- The highest sensitivity using a Polaroid® camera is achieved using Polaroid® 667 black-and-white print film and 490 nm longpass filter. Set the f-stop at 4.5 and expose for 1 second.
- The highest sensitivity using a CCD camera is achieved using 1024 x 1024 pixels resolution with 12- or 16-bit gray scale levels per pixel. Contact the camera's manufacturer for recommendations on which filter sets to use. **NOTE:** These parameters can vary depending upon the type of transilluminator being used.
- Laser scanning instruments that emit at 450 nm, 473 nm, 488 nm, or 532 nm can be used to visualize gels stained with SYPRO® Ruby Protein Blot Stain.

Post Blotting Analysis

Since SYPRO® Ruby Protein Blot Stain does not bind covalently to proteins, immunostaining, glycoprotein staining or mass spectrometry can be performed immediately after staining. Since over 90% of SYPRO® Ruby Protein Blot Stain is washed off the blot during the blocking step of immunostaining, it is important to document the staining prior to beginning the immunostaig procedure. If performing Edman-based sequencing subsequent to blotting, use a PVDF membrane, stain with SYPRO® Ruby Protein Blot Stain as above and then perform the following destain procedure:

1. Place the membrane face down in a solution of 150 mM Tris (pH 8.8)/20% methanol for 10 minutes using gentle agitation
2. Rinse the blot four times for 1 minute each in dH_2O
3. Air dry the membrane

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SYPRO® Stain Troubleshooting Guide

Symptom	Causes	Solution/Explanation
No Bands seen on gel	Wrong UV light box	Use a UV light box with 300 nm-312 nm lamps. Be sure the lamps are producing appropriate intensity.
	Very little protein	At the lower end of SYPRO® Stain's sensitivity range, the protein bands may not be visible by eye, but will show in the photograph.
	Incorrect stain dilution	Diluting the stain below the recommended concentration will result in reduced staining sensitivity.
	Use of colored stains, marker dyes or prestained protein markers	Colored stains or marker dyes, as well as commercially prestained protein markers, may interfere with SYPRO® Red staining and quench fluorescence. Use unstained markers.
	Stain container	Clean and rinse the staining dishes well before use as the detergent will interfere with staining. We recommend pipette-tip lid boxes, Rubbermaid® Servin'Saver® or Seal-A-Meal® type bags.
	Old running buffer	SDS can precipitate out of the running buffer, decreasing the stain's ability to bind. Use either fresh running buffer, or add SDS concentrate to buffer just prior to use.
	Destaining the gel	SYPRO® Stains do not require destaining. If required refer to destaining procedures.
No bands in photograph	High background	Use the correct stain dilution. A higher staining concentration can result in increased background and quench fluorescence.
	High background	The high background can sometimes obscure the protein bands. Use of the proper filters during photography will decrease background, allowing the bands to be visible. Decreasing the gel's SDS concentration to 0.05% from 0.1% may also help reduce background.
	Wrong exposure	Vary exposure until the background is low and the protein bands are visible. See protocol for recommended exposures.
	Improper photographic filters	Use the recommended photographic filters. For systems that use Wratten® filters, either the SYPRO® Filter or a Wratten #9 works best. Use a Tiffen® #15 (yellow-15) filter for systems that require threaded glass filters. For CCD cameras, consult the manufacturer for the appropriate filter (see protocol for excitation and emission maxima).
Bands visible on light box, but fade with time	Photobleaching of SYPRO® Stains	Restain the gel in fresh stain in the appropriate staining buffer.
No bands visible after staining in transfer buffer	Wrong methanol concentration	Use recommended methanol concentration in transfer buffer/stain. High methanol concentrations can strip SDS from the proteins, resulting in low detection levels.
No SYPRO® stained bands visible on Western blot after transfer	Proteins with low hydrophobicity	Only highly hydrophobic proteins will retain enough SYPRO® Stain to be visible on a membrane. SDS is stripped off proteins during transfer, resulting in very little retention of the SYPRO® Stain on most proteins.
Large blob of stain at bottom of gel	Binding of SYPRO® Stains to SDS front.	Run gel longer, or if running small proteins, change to Tris-Tricine buffer system.
Proteins not visible on native gels	SYPRO Stains require SDS/protein complexes to bind to proteins	Refer to procedure for staining nondenaturing proteins.
Protein not visible on Phast™ System gels or gels backed with GelBond® PAG Film	SYPRO® Stain binding to GelBond Film, which strongly autofluoresces	Remove GelBond® Film from the gel prior to staining. Use Clare Chemical's Dark Reader® transilluminator to image gels.

For more information contact Technical Service at (800) 521-0390 or visit our website at www.Lonza.com

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