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SYPRO® Ruby Protein Gel Stain

Introduction

SYPRO® Ruby Protein Gel Stain is a highly sensitive fluorescent stain for the rapid detection of proteins in polyacrylamide gels. This stain provides excellent sensitivity for standard 1-D SDS-PAGE, 2-D PAGE, and isoelectric focusing (IEF). The detection sensitivity **exceeds** Coomassie® Brilliant Blue Stain and **provides comparable sensitivity to that of the best silver staining techniques** (1ng-2 ng per band). SYPRO® Ruby Stain has the following advantages over silver staining:

- Simple staining protocol
- Less protein to protein variability
- Stains a wide variety of difficult–to-stain proteins including lipoproteins, calcium binding proteins, glycoproteins and fibrillar proteins
- No possibility of overstaining
- Does not interfere with mass spectrometry or Edman-based sequencing
- Provides a linear quantitation range of over three orders of magnitude

SYPRO® Ruby Stain can be used with a variety of gels including Tris-Glycine, Tris-Tricine, and nondenaturing.

Contents

SYPRO® Ruby Stain is available in three sizes and supplied ready to use. The three sizes provide enough material to stain the following number of gels:

- **200 ml** - ~ 4 minigels
- **1 L** - ~ 20 minigels or 2-3 large format gels
- **5 L** - ~ 100 minigels or 10-15 large format gels

Storage and Handling

SYPRO® Ruby Stain should be stored at room temperature and protected from light. Under these conditions, the stain is stable for at least 9 months.

When using the 5 L size, store the box on its side with top flap closed in order to protect the stain from light.

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** – An orbital shaker step at ~ 50 rmp 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** – 8x10 photographic trays work well.
- **Containers should be clean** – Rinse containers with ethanol prior to use.
- **Use circular staining dishes for staining small gels** – The use of these containers results in less dye aggregation and better staining.
- **Glass dishes are <u>not</u> recommended.**

General Procedure for Staining Proteins with SYPRO® Protein Gel Stains

1. Run gels according to standard protocols.
2. Fix the gel (***NOTE:*** fixation is not required for 1-D SDS polyacrylamide gels). Any of the following solutions can be used to fix 2-D gels. Fix for 20 minutes:

    - 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

NOTE: Combining ethanol and acetic acid can result in the formation of the toxic compound ethyl acetate which may interfere with identification of proteins via mass spectroscopy).

Fix IEF gels in a 40% methanol/10% trichloroacetic acid solution for three hours followed by three, ten minute washes in dH_2O.

3. Place the gel into the staining container and cover with a lid to protect from light. The container can also be wrapped in aluminum foil to further shield the stain from light during the staining process. The following are the minimum volumes that should be used to stain a gel of a given size:
    - 50 ml/8 cm x 10 cm x 0.75 mm gel
    - 330 ml/16 cm x 20 cm x 1 mm gel
    - 500 ml/20 cm x 20 cm x 1 mm gel
    - Approximately 10 times the volume of a given gel is needed for efficient staining.

4. Gently agitate the gel at room temperature.
5. Stain 1-D and 2-D gels for a minimum of three hours. Stain IEF gels overnight.
    - The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.
    - Increasing the staining time can increase sensitivity.
    - Gels will not overstain.
    - Diluting the stain from the concentration at which it is supplied will decrease sensitivity.
    - Reuse of the staining solution will result in a significant loss of sensitivity.
6. Transfer the gel to a clean staining dish and wash with a 10% methanol (or ethanol)/7% acetic acid solution for 30 minutes. This will minimize background fluorescence. IEF gels require this procedure to be repeated three times in order to achieve the best signal to background ratio. Gels can be monitored using UV epi-illumnination to determine the level of background fluorescence.
7. Remove the gel from the staining container and photograph the gel following the procedures outlined below.
8. The gel can be dried for permanent storage; however, proteins present at very low levels may no longer be detectable after drying. Incubate the gel in a 2% glycerol solution for 30 minutes prior to drying it using a gel dryer.

Photographing Gels Stained with SYPRO® Protein Gel Stains

SYPRO® Ruby Stain has two excitation maxima (at ~280 nm and ~450 nm), and one emission maximum (at ~610 nm). The stained gels can be visualized using a 300 nm UV transilluminator, a blue light transilluminator, or a laser scanner. SYPRO® Ruby Stain remains photostable 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 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 **NOTE:** These parameters can vary depending upon the for recommendations on which filter sets to use type of transilluminator being used.
- The backing on some precast gels is highly fluorescent. When using gels with a backing such as this, place the gel acrylamide side down on the transilluminator and use an emission filter to screen out the blue fluorescence of the plastic.
- Laser scanning instruments that emit at 450 nm, 473 nm, 488, or 532 nm can be used to visualize gels stained with SYPRO® Ruby Stain.

Identification of Individual protein spots

Since SYPRO® Ruby Stain does not bind covalently to proteins, it can be used in conjunction with Edman-based sequencing or mass spectroscopy after staining with no subsequent interference from the stain.

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 and Orange's staining and may 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.

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