SYPRO® Ruby Protein Stains

Instruction Manual

SYPRO® Ruby protein gel stain

Catalog Numbers

170-3125, 1 liter

170-3126, 200 ml

170-3138, 5 liter

SYPRO Ruby protein blot stain

Catalog Number

170-3127, 200 ml

SYPRO Ruby IEF stain

Catalog Number

170-3148, 400 ml

BIO-RAD

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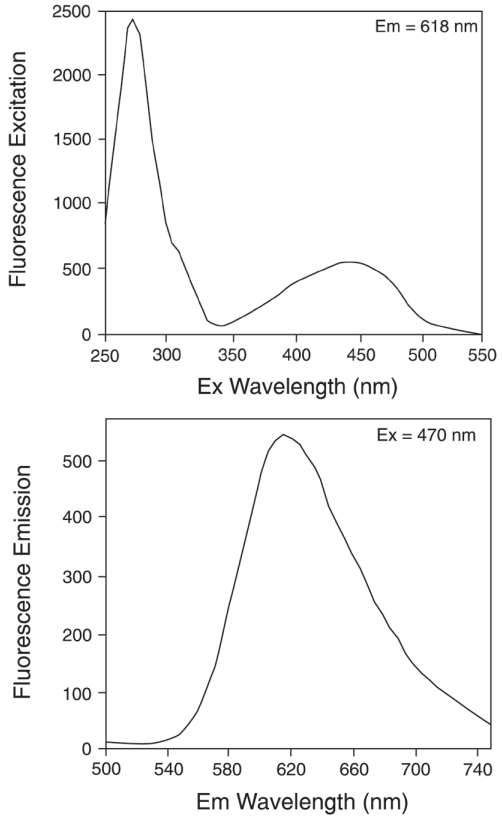
PART 1: Introduction to SYPRO Ruby protein stains

Section 1
General Information

1.1 Introduction

The fluorescent stain, SYPRO Ruby protein stain is available in three application specific formulations: 1) the SYPRO Ruby protein gel stain for staining proteins in acrylamide gels (1-D, 2-D, isolectric focusing); 2) the SYPRO Ruby protein blot stain for staining proteins on nitrocellulose or PVDF membranes; 3) the SYPRO Ruby IEF stain for staining proteins in acrylamide gels separated by isoelectric focusing (IEF). These formulations have been optimized specifically for each application and the stains are not interchangeable. Please refer to the appropriate section of this manual for the stain you are using.

1.2 Excitation and Emission



Fluorescence excitation and emission spectra of SYPRO Ruby protein gel stain.

1.3 Storage

Stable for at least 6 months when stored at room temperature, protected from light.

1.4 Materials Required But Not Supplied

a) Staining containers: plastic, preferably polypropylene or Polyvinyl chloride (PVC) photographic staining trays, such as Photoquip Cesco-lite 8 x 10 inch, can be used for large-format gels. Glass dishes are not recommended.

b) UV or blue light transilluminator with Polaroid™ or CCD camera or a laser-scanning instrument.

1.5 Safety Considerations

No data are available on the toxicity of SYPRO Ruby protein stains. The dye is comprised of an organic component and a heavy metal component (ruthenium). The SYPRO Ruby IEF stain is an acidic solution, which may cause burns. Eye protection and gloves should be worn and general laboratory safety precautions followed while handling all SYPRO Ruby protein stain products.

1.6 Disposal Considerations

Laws governing the disposal of laboratory chemicals vary by region. Check local laws for the proper disposal of SYPRO Ruby protein stain products.

PART 2: SYPRO Ruby protein gel stain

Section 1
General Information

1.1 Introduction

SYPRO Ruby protein gel stain is a ready–to–use, sensitive, fluorescent stain for detecting proteins separated by polyacrylamide gel electrophoresis (PAGE). While specially formulated for analysis of proteins in 2-D polyacrylamide gels, SYPRO Ruby protein gel stain also stains 1D gels of different buffer compositions including Tris-Glycine-SDS, Tris-Tricine and non-denaturing PAGE as well as IEF gels. The stain does not interfere with subsequent analysis of proteins by Edman-based sequencing or mass spectrometry and is quantitative over three orders of magnitude. Use SYPRO Ruby protein gel to stain difficult to stain proteins such as glycoproteins and lipoproteins. This stain will not stain nucleic acids.

1.2 Product Description

SYPRO Ruby protein gel stain is a 1x pre-mixed solution, and comes in three package sizes with instructions. The 200 ml size will stain ~ 4 mini gels, the 1 L size will stain ~20 mini gels or 2–3 large gels, the 5 L size will stain ~ 100 minigels or 10–15 large gels.

170-3125 SYPRO Ruby protein gel stain, 1 L
170-3126 SYPRO Ruby protein gel stain, 200 ml
170-3138 SYPRO Ruby gel stain, 5 L

Section 2
Instructions

2.1 Staining

a) Remove the gel from the gel cassette or plates. Place it in a clean plastic container (see Section1.4). For 2-D gels, wash for 30 min in one of the following gel fixing 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

For IEF gels, fix gel in 40% methanol, 10% trichloroacetic acid for 3 hours, then wash gel in water 3 times for 10 min each.

(No fixation is required for 1-D gels).

b) Remove the wash solution and cover the gel with SYPRO Ruby protein gel stain. The volumes used for typical gels are given below. In general use ~ 10 times the volume of the gel. Using too little stain will reduce sensitivity.

Gel Size	Volume of Stain
8 x 10 cm	50 ml
16 x 20 cm	330 ml
20 x 20 cm	500 ml

c) Stain the gel with continuous gentle agitation for at least 3 hrs for maximal sensitivity. Specific staining can be seen in 30–90 min. For convenience, gels may be left in the stain solution overnight (16–18 hrs) without overstaining.

d) Rinse the gel in 10% methanol (or ethanol), 7% acetic acid for 30–60 min. This rinse step decreases background fluorescence.

e) Wash gel in water before imaging.

2.2 Viewing and Imaging a 1D or 2D Gel

SYPRO Ruby protein gel stain has two excitation peaks at ~ 280 nm and ~ 450 nm and has an emission maxima near 610 nm. Stained proteins can be visualized using variety of excitation sources including a 300 nm UV or blue-light transilluminator, or laser-based systems. SYPRO Ruby protein gel stain also has exceptional photostability and a long emission lifetime, allowing for long exposure times while minimizing background fluorescence. For the superior resolution required for 2D gel analysis, the Bio-Rad Molecular Imager™ FX or Fluor-S™ MultiImagers are recommended.

a) UV or blue-light transilluminator

Gels that contain 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 with these systems.

- It is important to clean the surface of the transilluminator before each use with deionized water and a soft, lint free cloth to remove stains from the glass (including SYPRO, SYBR® and EtBr stain) that can lead to high background or skewed data.
- When capturing an image using a standard Polaroid camera system or CCD-based systems, a yellow filter such as the yellow Wratten 9 must be utilized for image collection. Typical Polaroid camera settings for capturing an image of a minigel stained with SYPRO Ruby protein gel stain are f/4.5 or better at a 1-second exposure setting.
- The Bio-Rad Gel Doc™ 1000, 2000, and ChemiDoc™ CCD-based camera systems are equipped with a UV transilluminator for imaging SYPRO Ruby protein gel stain.
- Bio-Rad's Fluor-S™ and Fluor-S MAX MultiImagers are equipped with trans-scanning UV for visualizing and imaging SYPRO Ruby stained gels. Use the Transmission Mode and 550LP filter.
- For other Polaroid-based or CCD-based systems please contact your camera manufacturer for appropriate recommendations on use of filter sets with SYPRO Ruby protein gel stain.

b) Laser-based instruments

- SYPRO Ruby protein gel stain can be imaged using laser-based imaging systems equipped with 450, 473, 488 or 532 nm laser lines. The following laser/filter combinations are recommended: 488/550LP; 488/605DF50; 532/550LP; 532/605DF50. The Bio-Rad Molecular Imager™ FX also has a pre-programmed function; select Protein Stain Gel/SYPRO Ruby from the Application Menu to automatically select the correct laser and filter sets for this stain.
- For other laser-based imaging systems please contact the manufacturer of the instrument for recommended use of filter sets with SYPRO Ruby protein stain.

PART 3: SYPRO Ruby protein blot stain

Section 1 Introduction

1.1 General Information

SYPRO Ruby protein blot stain is a ready-to-use, sensitive, fluorescent stain for detecting proteins on nitrocellulose or polyvinylidene difluoride (PVDF) membranes. SYPRO Ruby protein blot stain is a permanent protein stain that can be used to stain proteins on membrane supports prior to antibody- or lectin- mediated visualization of specific proteins. The stain can also be used prior to protein identification by Edman-based sequencing or Mass Spectrometry. Since SYPRO Ruby protein blot stain does not block epitopes, it can be used in conjunction with standard immunological procedures, eliminating the need for duplicate blots. This compatibility is particularly advantageous in blots of 2-D gels, where precise localization of the targeted protein relative to other proteins is often difficult.

1.2 Product Description

SYPRO Ruby protein blot stain is a 1x pre-mixed solution, provided with instructions. The 200 ml volume is sufficient for staining 10–40 mini gel electroblots, or ~ 4 large-format electroblots.

170-3127 SYPRO Ruby protein blot stain, 200 ml

Section 2 Instructions

2.1 Staining

Perform all washing and staining steps at room temperature in a clean plastic container (see Part 1, Section 1.4), with continuous, gentle agitation (*e.g.* on an orbital shaker). **Use forceps to handle wet blots, since residue found on latex gloves may destroy the staining pattern. Once dry, membranes can be handled freely**.

a) Staining Nitrocellulose Membranes

1) After electroblotting proteins to a nitrocellulose membrane, completely immerse the membrane in 7% acetic acid, 10% methanol and incubate for 15 minutes.
2) Wash the membrane in deionized water 4 times for 5 minutes each.
3) Completely immerse the membrane in SYPRO Ruby protein blot stain for 15 minutes.
4) Wash the membrane in deionized water 4 to 6 times for 1 minute each to remove excess stain. The membrane may be monitored periodically using UV epi-illumination to determine the level of background fluorescence.
5) Membranes stained with SYPRO Ruby protein blot stain are best preserved by allowing membranes to air dry.

b) Staining PVDF Membranes

1) After electroblotting proteins to a PVDF membrane, allow the membrane to dry completely.
2) Float the membrane face down in 7% acetic acid, 10% methanol and incubate for 15 minutes.
3) Wash the membrane in deionized water 4 times for 5 minutes each.
4) Transfer the blot using forceps to a staining dish containing SYPRO Ruby protein blot stain.
5) Float the membrane in SYPRO Ruby protein blot stain for 15 minutes.
6) Wash the membrane in deionized water 2 to 3 times for 1 minute each to remove excess stain. The membrane may be monitored periodically using UV epi-illumination to determine the level of background fluorescence.
7) Membranes stained with SYPRO Ruby protein blot stain are best preserved by allowing membranes to air dry. **After air drying they can be handled without using forceps.**

2.2 Visualizing and Imaging a SYPRO Ruby stained blot

RUBY BLOT stain has two excitation peaks at ~ 280 nm and ~ 450 nm and has an emission maxima near 618 nm.

a) **Epi UV Illumination**

- When viewing blots stained with SYPRO Ruby protein blot stain, only one side of the membrane can be excited due to the non-transparency of the membrane material. Therefore, only epi-UV illumination can effectively be used to visualize or image the blots. Epi illuminating CCD-based imaging systems or a hand-held UV source centered at 300 nm can be used for excitation: When capturing images with an epi-UV illuminated CCD-based system, a yellow filter such as the yellow Wratten # 9 must be utilized for image collection.
- Bio-Rad's Fluor-S™ and Fluor-S MAX MultiImagers are equipped with epi-UV for visualizing and imaging SYPRO Ruby protein blot stain. Use Epi-UV illumination and 610LP emission filter.
- For other epi-UV illuminated CCD-based systems please contact your camera manufacturer for appropriate recommendations on use of filter sets with SYPRO Ruby protein stains.

b) **Laser-based Instruments**

- SYPRO Ruby protein blot stain can be imaged using laser-based imaging systems equipped with 450, 473, 488 or 532 nm laser lines that use epi illumination and collection. For imaging blots with SYPRO Ruby protein blot stain on the Bio-Rad Molecular Imager™ FX use either the 488 or the 532 laser in combination with the 640DF35 filter. For other epi illuminated laser-based imaging systems please contact the manufacturer of the instrument for recommended use of filter sets with SYPRO Ruby protein stains.

Part 4: SYPRO Ruby IEF stain

Section 1 Introduction

1.1 General Information

SYPRO Ruby IEF stain is a ready–to–use, sensitive, fluorescent stain for detecting proteins separated in isoelectric focusing (IEF) gels. The stain does not interfere with subsequent analysis of proteins by Edman-based sequencing or mass spectrometry and is quantitative over three orders of magnitude. Use SYPRO Ruby IEF stain for difficult to stain proteins such as glycoproteins and lipoproteins. This stain will not stain nucleic acids.

1.2 Product Description

SYPRO Ruby IEF stain is a 1x pre-mixed solution, supplied with instructions. The 400 ml size will stain ~10 IEF mini gels or 2 standard flat-bed IEF gels.

170-3148 SYPRO Ruby IEF stain, 400 ml

Section 2 Instructions

2.1 Staining

Perform all washing and staining steps at room temperature in a clean plastic container (see Part 1, Section 1.4), with continuous, gentle agitation (*e.g.* on an orbital shaker). Use plastic, rather than latex, gloves to reduce the amount of smudging on the gel.

Note: RUBY IEF Stain contains a fixative; no fixation is required.

a) Incubate the gel in SYPRO Ruby IEF stain overnight (12–18 hrs)

Gel Size	Volume of Stain
6 x 9 cm	40 ml
8 x 10 cm	50 ml
24.5 x 11 cm	200 ml

b) Wash the gel in deionized water 4 times for 30 min each to decrease background fluorescence. The gel may be monitored periodically using UV epi-illumination to determine the level of background fluorescence.

Note: IEF gels with plastic backings separate from their backing during this water wash. It is important to remove this backing as it has high inherent fluorescence.

2.2 Viewing and Imaging an IEF Gel

SYPRO Ruby protein stain is excited around its two excitation peaks at ~ 280 nm and ~ 450 nm and has an emission maxima at ~ 610 nm. Stained proteins can be visualized using a variety of excitation sources including a 300 nm UV or blue-light transilluminator, or laser-based systems. SYPRO Ruby protein stain also has exceptional photo-stability and a long emission lifetime, allowing for long exposure times while minimizing background fluorescence.

a) UV or blue-light transilluminator

Gels that contain proteins stained with SYPRO Ruby IEF 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 with these systems.

- It is important to clean the surface of the transilluminator before each use with deionized water and a soft, lint free cloth to remove stains from the glass (including SYPRO, SYBR and EtBr stain) that can lead to high background or skewed data.
- When capturing an image using a standard Polaroid camera system or CCD-based systems, a yellow filter such as the yellow Wratten 9 must be used. Typical Polaroid camera settings for capturing an image of a minigel stained with SYPRO Ruby IEF stain are f/4.5 or better at a 1-second exposure setting.
- Bio-Rad's Gel Doc™ 1000, 2000, and ChemiDoc™ CCD-based camera systems are equipped with a UV transilluminator for imaging SYPRO Ruby IEF stained gels.
- Bio-Rad's Fluor-S™ and Fluor-S MAX MultiImagers are equipped with trans-scanning UV for visualizing and imaging SYPRO Ruby IEF stain. Use the Transmission Mode and 550LP filter.

- For other Polaroid-based or CCD-based systems please contact your camera manufacturer for appropriate recommendations on use of filter sets with SYPRO Ruby protein stain.

b) Laser-based instruments

- SYPRO Ruby IEF stain can be imaged using laser-based imaging systems equipped with 450, 473, 488 or 532 nm laser lines. The following laser/filter combinations are recommended: 488/550LP; 488/605DF50; 532/550LP; 532/605DF50. The Bio-Rad Molecular Imager™ FX laser-based system has a pre-programmed function for imaging this stain. Select Protein stain Gel/SYPRO Ruby from the Application Menu to automatically select the correct laser and filter sets for this stain.
- For other laser-based imaging systems please contact the manufacturer of the instrument for recommended use of filter sets with SYPRO Ruby protein stain.

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