3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

# ProductInformation

**SYPRO® Ruby Protein Gel Stain**

Product Number **S 4942**
Store at Room Temperature

## Product Description

SYPRO® Ruby Protein Gel Stain is an organometallic ruthenium chelate stain designed for proteomics applications. It is a sensitive and versatile protein stain for easy and simple detection of proteins in 1D and 2D SDS-polyacrylamide electrophoresis gels and in IEF gels. After staining proteins can be removed from the gel and analyzed by post-electrophoretic procedures, such as Edman-based sequencing and mass spectrometry without interference from the stain.[1,2]

SYPRO Ruby will detect glycoproteins, calcium-binding proteins, lipoproteins, fibrillar proteins, and other difficultly stained proteins without staining nucleic acids.[1] As little as 1-2 ng protein/band can be detected, similar to that of silver staining, but greater sensitivity than Coomassie® Brilliant Blue.[1-3]

The staining procedure is easy and ideal for high throughput. Gels cannot be overstained. After a quick rinse in 7.5% acetic acid gels can be photographed. Use a blue-light transilluminator, UV transilluminator, or laser scanner for detection. Fluorescence intensity is linear over three orders of magnitude of protein quantity, greater than either silver stain or Coomassie. Compared with silver stain the patterns obtained with SYPRO Ruby are similar, but not identical.[3]

Initially intended for 2D electrophoresis applications, SYPRO Ruby can also be used to stain Tris-glycine SDS-polyacrylamide electrophoresis gels and IEF gels.[4] SYPRO Ruby is compatible with gels attached to plastic backings.

SYPRO Ruby does not covalently bind to proteins. It associates with primary amines in proteins at acidic pH. Strongest interaction is with Lys, Arg, and His residues and less strongly with Tyr and Trp residues.[4]

The detection limit of SYPRO Ruby can be as low as 1 ng/band or approximately 0.12 ng/mm$^2$. The most sensitive detection is obtained using a photographic camera, CCD, or laser scanner image analysis system.

The dye has optimal excitation at 302 and 470 nm with an emission maximum at approximately 610 nm. A 300 nm UV or blue-light transilluminator, or laser scanner (488 nm or 532 nm, both with a 640 nm band pass filter) is used for detection.

SYPRO Ruby Protein Gel Stain does not interfere with later applications for the proteins. After documenting the stained gel may be treated with protease to prepare the samples for matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.[2,5] Proteins from stained gels can also be analyzed by Edman microsequencing procedures.[5]

## Reagent

SYPRO Ruby Protein Gel Stain is provided as a ready-to-use solution. For optimal sensitivity further dilution is not necessary or recommended.

## Storage/Stability

Store at room temperature protected from light. The solution is stable at least nine months.

## Procedure

Gel Staining

1. Fixing the gel:
   a. 2D gels: fix gels for 30 minutes in a solution of methanol or ethanol and acetic or trichloroacetic acid. Examples are 10% methanol with 7% acetic acid; 25% ethanol with 12.5% trichloroacetic acid; or 50% ethanol with 3% acetic acid.
   b. IEF gels: fix for three hours in a solution of 40% methanol with 10% trichloroacetic acid. Wash three time in water for 10 minutes each.
   c. 1D gels: Fixing not required before staining.
2. Pour stain into a clean, detergent-free polypropylene or polyvinyl chloride staining dish. Do not use glass dishes. Use approximately 50 ml for 8 × 10 cm (0.57 mm thick) gels to 500 ml for 20 × 20 cm (1 mm thick) gels. For larger gels use a volume of staining solution equal to approximately 10 times the gel volume. Using too little staining solution can reduce sensitivity.

3. Place gel in staining solution and cover with aluminum foil to protect from light during staining.
4. Place on a platform shaker for gentle agitation for at least three hours for 1D and 2D gels. IEF gels should be stained overnight. Gels will not overstain if left in staining solution overnight. Reuse of staining solution is not recommended because significant loss of sensitivity will result.
5. Washing the gels:
   a. Transfer gel to a clean staining dish. Wash gels in 10% methanol or ethanol containing 7% acetic acid for 30 minutes. IEF gels should be washed three times. Transferring to a new dish reduces fluorescent specks on the gel and washing improves sensitivity by reducing background fluorescence.
   b. Alternatively gels may be washed in water, but background fluorescence is not reduced as much.
6. Place gel directly on the transilluminator for photographing. Do not use plastic wrap because it will autofluoresce more than normal in the presence of SYPRO Ruby. If the gel has a plastic backing it should be removed if it autofluoresces. It may also bind the dye resulting in high background.

Detection:
SYPRO Ruby has excitation maxima at approximately 280 nm and 450 nm. For detection either a 300-nm UV transilluminator or a blue-light transilluminator can be used. Laser imaging systems with emissions at 450, 473, 488, or 532 nm can also be used.

Photography:
A photographic or CCD camera with appropriate filters attached will provide the greatest sensitivity.

For photography use Polaroid Type 667 (Product No. F 4638) or Type 57 (Product No. F 4513) film and the SYPRO Photographic Filter (Product No. S 6067). As a starting exposure use an f-stop of 4.5 and an exposure time of 1 second. Adjust these settings as needed to obtain optimal results. Transilluminators may have different light intensities depending on brand of instrument and age of bulbs. Other film types have lower film speeds requiring much longer exposures and possibly a different filter. Long exposures under UV illumination can be made, if needed, because SYPRO Ruby is very photostable.

**References**


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