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ProductInformation

SYPRO Ruby Protein Blot Stain

Product Number **S 4817**
Store at Room Temperature

Product Description

SYPRO Ruby Protein Blot Stain is an organometallic ruthenium chelate stain for detection of immobilized proteins on membranes (blots). It works with both nitrocellulose and polyvinylidene difluoride (PVDF) membranes.

SYPRO Ruby Blot Stain is selective for proteins. It does not interact with nucleic acids. Protein detection can be as low as 2-8 ng/band, equivalent to about 0.25-1.0 ng protein/mm^2. This compares with the sensitivity of colloidal gold stains, but staining can be done more quickly. It is about 25 times more sensitive than Amido Black and Coomassie® Brilliant Blue, and 50-60 times more sensitive than Ponceau S.

Unlike Amido Black and Coomassie Brilliant Blue, SYPRO Ruby Blot Stain will not covalently bond or otherwise modify proteins. Because it is compatible with other fluorogenic and chemiluminescent stains, it can be used in multiple staining techniques.[2] It will not interfere if the protein is to be used in later analytical procedures.

SYPRO Ruby can be used to stain proteins transferred to nitrocellulose or PVDF membranes by Western or electroblotting. Staining is permanent in most situations, but partial destaining can be achieved for post-staining analyses. The stain is fast and simple to use. The procedure can be completed in about an hour.

SYPRO Ruby Protein Blot Stain does not covalently bind to proteins. It does not block epitopes that may be useful in later immunostaining procedures.[3] Proteins can be analyzed by matrix-assisted laser desorption ionization (MALDI) and Edman microsequencing without interference from the stain.

The staining procedure is simple and faster than conventional methods such as colloidal gold. SYPRO Ruby staining is completed in an hour or less. Membranes are treated in an acetic acid/methanol solution, washed and placed in stain for 15 minutes. After washing away the excess stain solution blots are dried and ready for documentation and post-staining procedures.

Reagents

SYPRO Ruby Protein Blot Stain is provided as a ready-to-use solution. For optimal sensitivity further dilution is not necessary or recommended. One bottle (200 ml) will stain 10-40 minigel electroblots or four larger (20 cm × 20 cm) electroblots.

Storage/Stability

Store at room temperature protected from light. Stock solutions should be stable up one year.

Procedure

Nitrocellulose Blots:
Perform all steps with continuous gentle agitation. An orbital shaker set at 50 rpm is suggested. While membranes are wet they should be handled with forceps. Residues on latex gloves could destroy the staining pattern. Stained blots can be preserved by air drying. Once blots are dry it matters less how they are handled.

1. Upon completion of electroblotting, completely immerse the nitrocellulose membrane in a solution of 7% acetic acid with 10% methanol in a polypropylene staining dish. Incubate 15 minutes at room temperature.
2. Wash the membrane four times with water, each time for five minutes at room temperature.
3. Immerse the membrane completely in SYPRO Ruby Protein Blot Stain for 15 minutes.
4. Wash the membrane four to six times, one minute each time, in water.

PVDF Blots:
All staining and washing steps should be performed with the membrane floating face down on the surface of the solution and gently shaking on a platform shaker set at 50 rpm. While membranes are wet they should be handled with forceps. Residues on latex gloves could destroy the staining pattern. Once blots are dry it matters less how they are handled.

1. After blotting is complete allow the membrane to dry completely.
2. Place the membrane face down on the surface of a solution of 7% acetic acid with 10% methanol and agitate for 15 minutes.
3. Wash the membrane four times by placing face down on the surface of water for five minutes.
4. Place the membrane face down on the surface of the SYPRO Ruby Protein Blot Stain for 15 minutes.
5. Remove the excess dye from the membrane by washing with three changes of water for one minute each wash.
6. Preserve the stained blot by air drying.

Detection:
SYPRO Ruby Blot Stain has excitation maxima at approximately 280 nm and 450 nm with emission at 618 nm. For detection a 300-nm UV-B transilluminator or a blue-light fluorescent or blue-light LED transilluminator can be used. Laser imaging systems with emissions at 488 nm (argon) or 532 nm (yttrium-aluminum-garnet) can also be used.[1]

Visual detection of the stained blot can be done using a hand held UV-B light source or a UV transilluminator placed on its side. To photograph the blot, place it directly on the transilluminator.

Photography:
The use of 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 film (Product No. F 4513) 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 from these settings as needed to obtain optimum results. Transilluminators may have different light intensities depending on brand and age of bulbs. Other film types have lower film speeds requiring much longer exposures and possibly a different filter.

Post-staining analysis:
1. Stained blots can be subjected to other staining procedures such as immunostaining and glycoprotein staining. It is important to document the SYPRO Ruby stained gel by photography or other means prior to other staining. Washing steps in immunostaining procedures can cause significant loss of SYPRO Ruby.
2. Stained proteins can be trypsin digested and analyzed by MALDI mass spectrometry.[1]
3. For Edman microsequencing blots should be prepared on PVDF membranes. After staining the blot with SYPRO Ruby blot stain and documenting, the blot should be partially destained.
   a. Place blot face down on a solution of 150 mM Tris, pH 8.8 with 20% methanol for ten minutes with gentle shaking.
   b. Rinse blot in water four times for one minute each time.
   c. Air dry.

References

1. Berggren, K., et al., A luminescent ruthenium complex for ultrasensitive detection of proteins immobilized on membrane supports. Anal. Biochem., **276,** 129-143 (1999).
2. Ducret, A., et al., A general method for the rapid characterization of tyrosine-phosphorylated proteins by mini two-dimensional gel electrophoresis. Electrophoresis, **21,** 2196-2208 (2000).
3. Neumann, H., and Mullner, S., Two replica blotting methods for fast immunological analysis of common proteins in two-dimensional electrophoresis. Electrophoresis, **19,** 752-7 (1998).

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