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ProductInformation

SYPRO Red Protein Gel Stain

Product Number **S 5817**
Store at Room Temperature

Product Description

SYPRO Red Protein Gel Stain is a sensitive protein stain for fast and simple detection of proteins in electrophoresis gels.

1D SDS-PAGE protein staining is non-selective, but specific. Under denaturing conditions the dye associates with the SDS-protein micelles and not directly with the protein. Bacterial lipopolysaccharides are only weakly stained and nucleic acids are not stained. This specificity gives SYPRO Red an advantage over the more traditional silver stain while providing the same sensitivity. SYPRO Red can detect as little as 4-8 ng protein/band and molecular weights down to about 6.5 kDa, similar to that of silver staining but with greater sensitivity than Coomassie® Brilliant Blue.

The staining procedure is fast and simple. No prior fixing is necessary. After staining for 30-60 minutes in the dye solution, gels can be photographed after a quick rinse in 7.5% acetic acid solution. Place on a 300 nm UV transilluminator or laser scanner for detection. Fluorescence intensity is linear over three orders of magnitude protein quantity, greater than either silver stain or Coomassie.

SYPRO Red can be added to the cathode running buffer for staining proteins while the gel is running. The dye runs with the SDS front and stains all proteins. Proteins cannot be pre-stained because the SDS in the denaturing buffer will interfere. Proteins can be detected on non-denaturing gels after soaking in a solution of SDS to prepare the protein for staining. If SYPRO Red is used to stain 2D and IEF gels sensitivity will be greatly reduced. We recommend SYPRO Ruby Protein Gel Stain (Product No. S 4942) for these procedures.

SYPRO Red stains by interacting with the SDS associated with the denatured protein. Running buffers should contain no more that 0.05% SDS. This concentration does not affect resolution and permits faster protein staining. For optimal results SDS stock solutions and running buffers should be freshly prepared.

The staining solution may be used up to four times, but sensitivity is reduced with each subsequent use because of loss of dye.

If proteins of interest are very small or a low gel percentage is used, better protein retention is achieved if the acetic acid concentration in the staining solution is increased to 10%. Sensitivity will not be affected.

Gels containing acetic acid are not suitable for Western blots or other blotting techniques. Acetic acid interferes with the transfer process. SYPRO Red can be diluted in a transfer buffer for this purpose, but sensitivity may be adversely affected. We recommend either to stain with SYPRO Tangerine Protein Gel Stain (Product No. S 5942), which does not use acetic acid for fixing or complete the transfer and stain the blot with SYPRO Ruby Protein Blot Stain (Product No. S 4817).

TRITON® X-100 interferes with staining at concentrations above 0.1%. If used in electrophoresis it will be necessary to dilute the Triton X-100 with three washes of buffer followed by a soaking with 0.05% SDS before staining.

Reagent

SYPRO Red Protein Gel Stain is provided as a 5000x concentrated solution in DMSO.

Preparation Instructions
To stain after electrophoresis dilute the stock solution 5000-fold in 7.5% (v/v) acetic acid solution with vigorous mixing. A 50 µl aliquot of stock solution prepares enough working solution to stain five polyacrylamide minigels.

For staining during electrophoresis dilute the stock solution 5000-fold in cathode running buffer.

Storage/Stability
Store desiccated at room temperature.
Protect from light.

Stock solutions should be stable up to a year when stored protected from light at room temperature, 4 °C, or –20 °C. Solutions diluted in buffer or dilute acetic acid should be stored in clean, detergent-free glass or plastic bottles, protected from light, at 4 °C for three months.

Note: if particles of dye are present in the stock solution after is comes to room temperature sonicate briefly or vigorously vortex to redissolve.

Procedure
Staining after electrophoresis:
1. Pour 1x staining solution into a clean, detergent-free glass or polypropylene staining dish. Use about 50 ml for one or two standard minigels and up to 750 ml for larger gels.
2. Place gel in staining solution and cover with aluminum foil to protect from light during staining.
3. Gently agitate on a platform shaker for typically 40-60 minutes or until optimal staining is achieved. Extended staining times will not improve sensitivity, but can increase background fluorescence.
4. Rinse gel in 7.5% acetic acid solution for 1 minute to remove excess dye from the gel.
5. 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 Red. If the gel has a plastic backing it should be removed if it autofluoresces. The backing may bind the dye resulting in high background.
6. If a laser scanner is used best results are obtained if a He-Ne or Nd-YAG (yttrium-aluminum-garnet) laser-based instrument is used.

Staining During Electrophoresis
1. Dilute the stock solution 5000-fold in cathode running buffer. Do not use SDS at a concentration greater than 0.05%. This will maintain denaturing conditions with a minimal effect on sensitivity and background.
2. Add to apparatus after the samples have been loaded. Do not allow to mix because the SDS in the sample buffer will interact with the stain to decrease sensitivity.
3. Run gel in normal manner.
4. Destain gel in 7.5% acetic acid solution for 15-40 minutes to remove excess dye from the gel.

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 2-5 seconds. Adjust from these settings as needed to obtain optimum 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. Extended exposure to UV light (several minutes) can cause photobleaching. If photobleaching occurs the gel can be returned to the staining solution to restain.

References

1. Steinberg, T. H., et al., SYPRO orange and SYPRO red protein gel stains: one-step fluorescent staining of denaturing gels for detection of nanogram levels of protein. Anal. Biochem., **239,** 223-237 (1996).
2. Singer, V. L., et al., Highly Sensitive SYPRO Fluorescent Protein Gel Stains. J. NIH Res., **7,** 82 (1995).
3. Steinberg, T. H., et al., Applications of SYPRO orange and SYPRO red protein gel stains. Anal. Biochem., **239,** 238-245 (1996).
4. Steinberg, T. H., et al., Optimal filter combinations for photographing SYPRO orange or SYPRO red dye-stained gels. Anal. Biochem., **248,** 168-72 (1997).

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