

Product Information

SYPRO Tangerine Protein Gel Stain

Product Number **S 5942**
Store at Room Temperature

Product Description

SYPRO Tangerine Protein Gel Stain is a sensitive and versatile stain for fast and simple detection of proteins in electrophoresis gels. Staining can be done in saline. No acids or organic solvents are required. However, proteins can be stained with SYPRO Tangerine if gels are fixed in 7% acetic acid or 12.5% trichloroacetic acid solution.

1D SDS-PAGE protein staining is non-selective, but specific. Under denaturing conditions the dye associates with the SDS- protein micelles in the gel. It does not stain nucleic acids or lipopolysaccharides. This specificity gives SYPRO Tangerine an advantage over the more traditional silver stain while providing the same sensitivity. SYPRO Tangerine 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 more sensitive than Coomassie Brilliant Blue.

The staining procedure is fast and simple. No prior fixing is necessary. After staining for typically 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 of protein quantity, greater than either silver stain or Coomassie.

Because no hazardous materials are used for staining and detection can be made on a blue-light transilluminator (no UV exposure) SYPRO Tangerine is suggested for group demonstration gels.

SYPRO Tangerine is ideal if the proteins are to be used in post-electrophoresis procedures. The dye has no effect on the structure of the proteins. If SDS does not interfere with the activity of enzymes of interest they can be assayed by zymography. Gels can be used in blotting procedures or specific bands eluted for mass spectrometry.

If the proteins are not to be used in post-electrophoresis procedures gels can be fixed in 7.5% acetic acid solution. This step will improve band morphology and minimize diffusion, especially of smaller proteins.

SYPRO Tangerine is not recommended for IEF gels and provides only moderate sensitivity in 2D gels. SYPRO Ruby Protein Gel Stain (Product No. S 4942) is recommended for these procedures.

SYPRO Tangerine can be used to stain SDS-polyacrylamide gels that have been run in a variety of buffers over a range of pH values (4.0-10.0). Buffer concentrations should be 50-100 mM containing 150 mM NaCl. If post-electrophoresis procedures are to be used, a phosphate saline buffer pH 7.0 (50 mM phosphate, 150 mM NaCl, pH 7.0) is recommended.

If the gel is to be used to prepare a Western blot it can be first stained to detect the proteins. After staining prepare the blot using a standard Western buffer containing 0.1% SDS. While the SDS is not required it has made transfers of some proteins easier. Gels intended for blotting should not be fixed with acetic acid. Acetic acid interferes with the transfer process.

For non-blotting procedures fix the gel in 7.5% acetic acid solution to improve band morphology and minimize protein diffusion in the gel. For better retention of smaller proteins or low percentage gels fixing in 10% acetic acid solution is recommended. Sensitivity will not be adversely affected.

Fixing solutions should not contain methanol because the methanol tends to remove the SDS from the surface of the protein resulting in reduced sensitivity.

Staining with a dye concentration below the recommended concentration will result in reduced sensitivity. Staining at higher concentrations or for longer times than recommended will result in higher background with no significant increase in detection or sensitivity. Fluorescence may actually be quenched by the excess dye molecules.

Reagent

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

Preparation Instructions

If post-electrophoresis procedures are to be used the SYPRO Tangerine stock solution should be diluted 5000-fold in 50 mM phosphate buffer, pH 7.0, containing 150 mM NaCl, with vigorous mixing. Other compatible buffers include citrate, pH 4.5; MES, pH 6.0; Tris-acetate, pH 8.0; and carbonate, pH 10.0. A 50 μ l aliquot of stock solution prepares enough working solution to stain five polyacrylamide minigels.

Storage/Stability

Store desiccated at room temperature.
Protect from light.

Stock solutions should be stable at least six months when stored protected from light at room temperature, 4 °C, or -20 °C. Solutions diluted in buffer should be stored in clean, detergent-free glass or plastic bottles, protected from light, at 4 °C for at least three months.

Procedure

Staining after electrophoresis:

1. Pour stain 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. Place on a platform shaker for gentle agitation for 10-60 minutes or until optimal staining is achieved. Staining time is determined by polyacrylamide concentration and the thickness of the gel. Extended staining times will not improve sensitivity, but can increase background fluorescence.
4. 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 Tangerine. If the gel has a plastic backing it should be removed if it autofluoresces. The backing may bind the dye resulting in high background.

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., Fluorescence detection of proteins in sodium dodecyl sulfate-polyacrylamide gels using environmentally benign, nonfixative, saline solution. *Electrophoresis*, **21**, 497-508 (2000).
2. Lauber, W. M., et al. Mass spectrometry compatibility of two-dimensional gel protein stains. *Electrophoresis*, **22**, 906-18 (2001).

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