
SYPRO[®] Orange Protein Gel Stain Instruction Manual

**Catalog Number
170-3120**

Product shipped at room temperature
Store at 4 °C upon arrival

BIO-RAD

Introduction

SYPRO Orange protein stain is a fluorescent reagent for the rapid staining of proteins with high sensitivity and low background following SDS or native polyacrylamide gel electrophoresis. The stained proteins are visualized by ultraviolet illumination (about 302 nm) with standard transilluminators; alternatively, they may be visualized under 488-nm excitation. **In order to fully exploit the stain's sensitivity, the gel must be photographed or digitally imaged.** SYPRO Orange protein stain may be used to stain gels before western blotting, electro-elution, or protein sequencing. SYPRO Orange protein stain will not overstain proteins even after extended incubation. The stain is provided as a 5,000x concentrate in dimethyl sulfoxide (DMSO) and may be reused at least once. For optimal performance, staining should be carried out in plastic (polypropylene recommended) rather than glass trays.

The exact mechanism of the interaction between proteins and SYPRO Orange protein stain has not been fully characterized, but the interaction is dependent upon an initial binding of SDS to the proteins. Proteins that are electrophoresed in the presence of SDS are ready for staining by SYPRO Orange protein stain immediately following electrophoresis. Proteins run in buffer systems without SDS, *i.e.*, native gels, must first be soaked in an SDS-containing solution before staining. To a lesser extent, free SDS can interact with SYPRO Orange protein stain

to cause background fluorescence. When proteins are electrophoresed in an SDS-containing buffer, most of the free SDS which contributes to background elutes from the gel during the staining step. If the staining solution contains SDS, then (equilibrium conditions will prevent sufficient SDS from leaching from the gel and) the gel will have very high levels of background, obscuring the signal of the stained proteins.

Physical Data

The excitation and emission spectra of SYPRO Orange protein stain are shown below in Figures 1 and 2. The data were collected on a 1:5,000 dilution of SYPRO Orange protein stain with 0.05% SDS and 150 $\mu\text{g/ml}$ BSA.

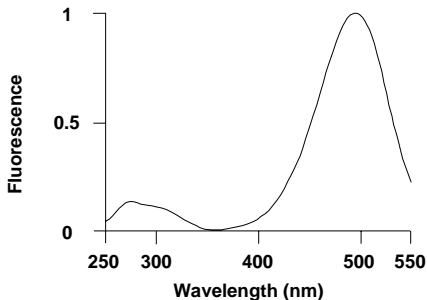


Fig. 1. SYPRO Orange protein stain excitation spectrum.

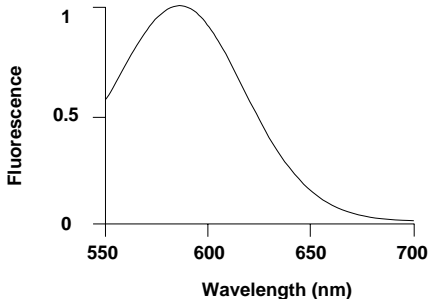


Fig. 2. SYPRO Orange protein stain emission spectrum.

SDS-PAGE

SYPRO Orange protein stain may be used following SDS-PAGE carried out with the standard Laemmli¹ buffer system. To make 1 liter of 5x running buffer, combine

- 15.0 g Tris base
- 72.0 g Glycine
- 5.0 g SDS
- 900 ml of ddH₂O

When the solids are dissolved, bring the final volume to 1 liter.

Make sample loading buffer by combining

- 4.0 ml ddH₂O
- 1.0 ml 0.5M Tris-HCl, pH 6.8
- 1.6 ml 10% (w/v) SDS
- 0.8 ml glycerol
- 0.2 ml 1% (w/v) bromophenol blue

Divide the sample buffer into 475 μ l aliquots and freeze until use. Immediately before using, add 25 μ l of fresh β -mercaptoethanol to the 475 μ l of sample buffer. Dilute protein samples at least 1:4 with sample buffer (*e.g.*, 3 μ l protein + 12 μ l sample buffer), and heat to 95 °C for 5 minutes before loading onto the gel.

Staining SDS-PAGE Gels

To obtain optimal detection sensitivity, stain the gel in a polypropylene (rather than glass) staining tray with a 1:5,000 dilution of SYPRO Orange protein stain in 7.5% (v/v) acetic acid. Staining in acetic acid will permanently fix the protein in the gel.

Strictly following protocol yields best results.

- For minigels, add 10 μ l of SYPRO Orange protein stain to 50 ml of 7.5% acetic acid in a plastic staining dish. For larger format gels, add 20 μ l

SYPRO Orange protein stain to 100 ml of 7.5% acetic acid.

- Mix the SYPRO Orange protein stain in the acetic acid thoroughly, then add the gel to the solution. Cover the incubation tray with aluminum foil to protect the stain from light and to prevent dust deposition on the gel.
- Incubate the gel in diluted stain for 30 minutes with gentle agitation at room temperature.
- After 30 minutes, remove the gel from the staining solution. Rinse the gel in fresh 7.5% acetic acid for exactly 30 seconds to remove excess staining solution from the surface of the gel. Exceeding 30 seconds in acetic acid solution may decrease sensitivity. (Do not discard the stain solution until the gel has been photographed or digitally imaged).
- Place the gel on the UV light box for photography or imaging. Dust particles on the surface in the gel will fluoresce, but these may be removed by squirting the gel with ddH₂O from a water bottle. Normally the background fluorescence is acceptable after 30 minutes in the staining solution, but if it is too high, return the gel to the staining solution for another 30–60 minutes. The proteins cannot be overstained by the extended incubation, but background will decrease as SDS elutes from the gel.

Documentation

The human eye is not as sensitive as film or CCD arrays to SYPRO Orange-stained proteins. Therefore, to take full advantage of the stain, the stained gel must be photographed with Polaroid® 667 black and white film, or it must be digitally imaged with a CCD-based instrument such as the Fluor-S™ MultiImager or the Gel Doc™ 2000 UV fluorescent gel documentation system. Photography or imaging must be done with an orange filter (*e.g.*, the Wratten 23A used for ethidium bromide-stained DNA gels) or a yellow filter (*e.g.*, Wratten 9; use the 520 LP filter with the Fluor-S MultiImager). With an orange filter, **typical film exposures are from 3 to 30 seconds, with an average of 15 seconds at f-stop 5.6. Typical integration on a CCD system ranges from 1 to 5 seconds, with an average of 2 seconds.** Yellow filters can halve the exposure/integration time. A good technique is to expose/integrate until background becomes just visible.

Alternative Protocols

Staining Before Western Blotting

For staining prior to western blotting or electro-elution, follow the protocol for SDS-PAGE, except substitute SDS-free transfer buffer (with or without 20% methanol) for the acetic acid. Sensitivity in the gel will be slightly

diminished by staining in the transfer buffer. Destaining prior to blotting is unnecessary as most of the stain will be removed during the washing and blocking steps of the detection procedure.

Staining Before Protein Sequencing

We have shown that proteins blotted to PVDF membranes following staining with SYPRO Orange protein stain may be successfully sequenced by Edman degradation in an automated instrument.² After electrophoresis optimized for subsequent protein sequencing,³ incubate the gel at room temperature for 30 minutes in a 1:5,000 dilution of SYPRO Orange protein stain in Towbin⁴ buffer. Photograph or image the gel if desired, and then blot the proteins to PVDF by standard techniques. Identify the location of the membrane-bound proteins by ultraviolet epi-illumination of the PVDF membrane. Use a pencil to circle the position of the protein, and then, in room light, cut out the appropriate section of the membrane. The bound protein may be sequenced by standard automated methods.

Staining Peptides

SYPRO Orange protein stain may be used to stain peptides down to 2,000 daltons following electrophoresis in a Tris/Tricine/SDS system.⁵ After electrophoresis, soak the gel in 10% acetic acid for 30–45 minutes to fix the peptides, then follow the protocol described for SDS-PAGE.

Staining Native Gels

Omit SDS from gels and running buffer made for the electrophoresis of native proteins. Prepare the sample loading buffer with water in place of the SDS and β -mercaptoethanol. Do not heat the samples before electrophoresis. After electrophoresis, soak the gel in 7.5% acetic acid + 0.05% SDS for 30 minutes before following the staining protocol described for SDS-PAGE. **Note:** The SDS soak and SYPRO Orange staining steps may be combined into one 30-minute incubation, but it must be followed by an additional 20–30 minute incubation in fresh 7.5% acetic acid to reduce background to acceptable levels. **Note:** Acetic acid will denature and fix the proteins in the gel. In order to maintain the native conformation and/or allow eventual recovery of the proteins, substitute SDS-free running or elution buffer for the acetic acid in all steps of the protocol.

Destaining

Gels may be partially destained by soaking in staining solution (*i.e.*, 7.5% acetic acid, running buffer or transfer buffer) without SYPRO Orange protein stain. A partially destained gel may be restained by addition of fresh SYPRO Orange protein stain.

Reuse of Stain

Dilute staining solutions may be reused at least once without loss of sensitivity. Store the dilute solution up to a week in a plastic (polypropylene recommended) container in the dark at 4 °C.

Storage and Handling

SYPRO Orange protein stain is guaranteed for 6 months when stored at 4 °C and protected from light. Completely thaw and then vortex the solution before use. DMSO should be handled with caution, and gloves are recommended.

Ordering Information

**Catalog
Number**

Product Description

Ordering Information

161-0424	Staining Box, 12x12 cm
161-0330	SYPRO Orange Standards, Low Range, 200 µl
161-0331	SYPRO Orange Standards, High Range, 200 µl
161-0332	SYPRO Orange Standards, Broad Range, 200 µl
170-3120	SYPRO Orange Concentrate, 500 µl

References

1. Laemmli, U. K., *Nature*, **227**, 680 (1970).
2. Hamby, R. K., *Am. Biotech. Lab.*, **14**, 12 (1996).
3. Speicher, D. W., in *Techniques in Protein Chemistry* (T. Hugli, ed.), Academic Press, 24–35. Bio-Rad PVDF Protein Sequencing Membrane Instruction Manual (1989).
4. Towbin, H., *et al.*, *Proc. Nat. Acad. Sci. USA*, **76**, 4350 (1979).
5. Schagger, H. and von Jagow, G., *Anal. Biochem.*, **166**, 368 (1987).

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