

SYPRO[®] Orange and SYPRO[®] Red Protein Gel Stains

Quick Facts

Storage upon receipt:

- Storage temperature not critical
- Desiccate
- Protect from light

Ex/Em:

- SYPRO Orange dye: 300, 470/570 nm
- SYPRO Red dye: 300, 550/630 nm

Introduction

Molecular Probes' proprietary SYPRO[®] Orange and SYPRO[®] Red protein gel stains provide fast, simple, sensitive staining of proteins in electrophoretic gels and offer the following advantages over conventional colorimetric stains:

- **High sensitivity.** SYPRO Orange and SYPRO Red protein gel stains can detect 4–8 ng of protein per minigel band, higher sensitivity than Coomassie[®] brilliant blue (CBB) staining and as sensitive as many silver staining techniques (Figure 1).
- **Rapid staining.** Staining is completed in less than an hour.^{1,2}
- **Simple staining procedure.** After electrophoresis, the gel is simply stained, rinsed and photographed — no separate fixation or destaining steps are required, and there is no fear of overstaining the gel.^{1,3}
- **Compatibility with standard laboratory equipment.** Stained proteins can be visualized using a standard 300 nm UV transilluminator or a laser scanner (Figure 2).
- **Low protein-to-protein variability.** Because SYPRO Orange and SYPRO Red dyes interact with the SDS coat around proteins in the gel, they give more consistent staining between different types of proteins compared to CBB staining¹ and never exhibit negative staining. SYPRO Orange and SYPRO Red stain glycoproteins well.
- **High selectivity for proteins.** SYPRO Orange and SYPRO Red protein gel stains detect a variety of proteins down to ~6500 daltons without staining nucleic acid or lipopolysaccharide contaminants that are sometimes found in protein preparations derived from cell or tissue extracts.
- **Broad linear range of detection.** The fluorescence intensity of SYPRO dye-stained bands is linear with protein quantity over three orders of magnitude, a much broader range than either CBB or silver staining can provide.¹

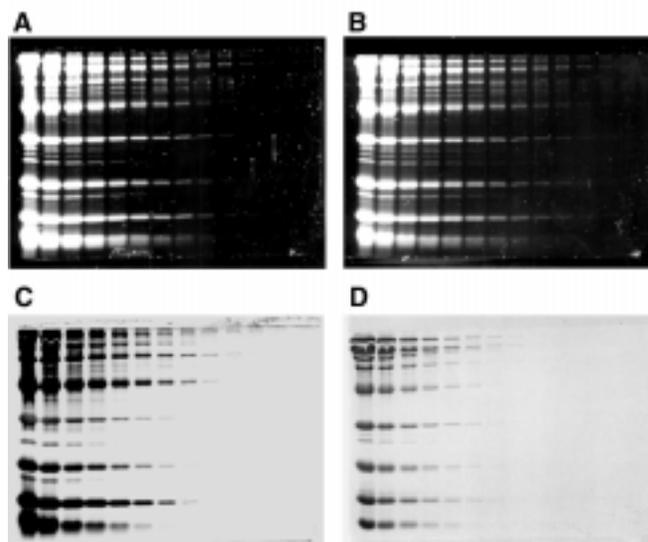


Figure 1. Identical polyacrylamide minigels stained with A) SYPRO Orange gel stain, B) SYPRO Red gel stain, C) silver stain and D) Coomassie brilliant blue (CBB) stain according to standard protocols. The SYPRO dye-stained gels were photographed using 300 nm transillumination, a SYPRO photographic filter (S-6656) and Polaroid 667 black-and-white print film. The CBB- and silver-stained gels were photographed using transmitted white light and Polaroid 667 black-and-white print film; no optical filter was used.

The staining properties of SYPRO Orange and SYPRO Red dyes are similar, and both are equally suitable for use in most procedures. The SYPRO Orange gel stain is slightly brighter, whereas the SYPRO Red gel stain has somewhat lower background fluorescence. Both dyes are efficiently excited by UV or broadband illumination (Figure 2) and, with the proper filters, work well with CCD camera archiving systems. For those using a laser scanner, choose the dye with the excitation maximum most closely matching the excitation light in the scanner (Figure 2).

SYPRO Orange and SYPRO Red protein gel stains are not suitable for staining proteins on blotting membranes and they show greatly reduced sensitivity when staining proteins in IEF or 2-D gels. For these applications, we recommend our SYPRO Ruby protein blot stain (S-11791), and SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900), respectively.

Materials

Contents

SYPRO Orange protein gel stain (S-6650, S-6651) and SYPRO Red protein gel stain (S-6653, S-6654) are provided as

5000X concentrated solutions in dimethylsulfoxide (DMSO), either as a single vial containing 500 μ L of stock solution or as a set of 10 vials, each containing 50 μ L of stock solution. In each case, enough reagent is supplied to prepare a total of 2.5 L of working stain solution, which is sufficient to stain ~50 polyacrylamide minigels.

We also offer the SYPRO Protein Gel Stain Starter Kit (S-12012), which includes:

- SYPRO Orange protein gel stain, 50 μ L
- SYPRO Red protein gel stain, 50 μ L
- SYPRO Tangerine protein gel stain (S-12010), 50 μ L
- SYPRO protein gel stain photographic filter (S-6656)

Storage

The SYPRO stock solutions should be stored protected from light at room temperature, 4°C or -20°C. When stored properly, these stock solutions are stable for six months to one year. The staining reagent diluted in buffer or acetic acid solution can be stored in very clean and detergent-free glass or plastic bottles, protected from light at 4°C for at least three months.

Handling

Before opening, each vial should be allowed to warm to room temperature and then briefly centrifuged in a microfuge to deposit the DMSO solution at the bottom of the vial. If particles

of dye are present, they should be redissolved by briefly sonicating the tube or vortexing it vigorously after warming.

Disposal

We must caution that no data are available addressing the toxicity of the SYPRO protein gel stains. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling DMSO stock solutions. Solutions of SYPRO stains should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dye. We have found that 1 g of activated charcoal binds at least 98% of the SYPRO Orange or SYPRO Red dye present in 2.5 L of 1X staining solution prepared in 7.5% acetic acid, which is equivalent to the amount of dye in 500 μ L of the 5000X concentrated DMSO solution.

SDS-Polyacrylamide Gel Electrophoresis

Prepare and run SDS-polyacrylamide gel according to standard protocols.^{4,5} For the highest sensitivity, we recommend using 0.05% SDS in the running buffer (instead of the usual 0.1% SDS). Gels run in 0.05% SDS show no change in the migration of proteins and can be photographed more quickly because they require less time in the staining solution to clear the SDS from the gel. Gels run in SDS concentrations lower than 0.05% or in old running buffer exhibit poor resolution of bands and other problems, so it is essential that the SDS stock solution used to prepare the running buffer be fresh and at the proper SDS concentration.

Staining Proteins in the Gel

Staining Proteins After Electrophoresis

1. Prepare the staining solution by diluting the stock SYPRO reagent 1:5000 in 7.5% (v/v) acetic acid and mixing vigorously.

- Diluting the stain below the recommended concentration will result in reduced staining sensitivity.
- Using higher staining concentrations than recommended will not result in better detection, but will instead result in increased background in the gel and quenching of the fluorescence from dye molecules crowded around the proteins.
- The staining solution may be reused up to four times. However, we see dramatically reduced sensitivity, especially after the second reuse and therefore recommend using fresh staining solution for optimal sensitivity.
- For low percentage gels and for very small proteins, 10% acetic acid solution will result in better retention of the protein in the gel without compromising sensitivity.
- Acetic acid will interfere with transfer of the proteins to a blot. For Western blotting and other blotting techniques, you may dilute the SYPRO Orange or SYPRO Red stain in standard transfer buffer. However, staining the gel in transfer buffer will result in lower sensitivity staining. Therefore, for blotting techniques, we recommend staining the gel with SYPRO Tangerine protein gel stain (S-12010), which does not require acetic acid fixation, or staining the blot directly with SYPRO Ruby protein blot stain (S-11791).

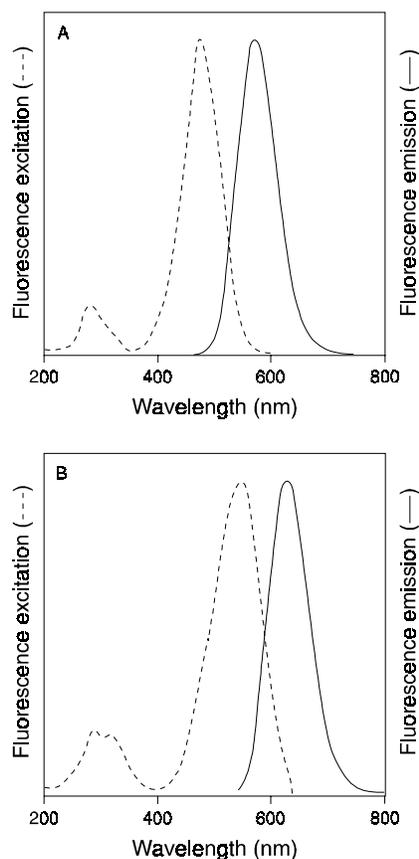


Figure 2. The fluorescence excitation and emission spectra of A) SYPRO Orange and B) SYPRO Red protein gel stains diluted 1:10,000 in water containing 0.05% SDS and 150 μ g/mL bovine serum albumin.

- Do not fix the proteins in the gel with methanol-containing solutions. Methanol removes the SDS coat from proteins, strongly reducing the signal from SYPRO Orange or SYPRO Red protein gel stains.

2. Pour the staining solution into a small plastic dish.

- For one or two standard-size minigels, use about 50 mL of staining solution. For larger gels, use between 500 and 750 mL of staining solution.
- We use Rubbermaid® Servin' Saver containers with lids, but also find that the lids of pipet boxes work just fine.
- Clean and rinse the staining dishes well before use as detergent will interfere with staining.
- Gels may also be stained in Seal-A-Meal® type bags. It is still important to use the proper amount of staining solution.

3. Place the gel into the staining solution.

- Cover the container with aluminum foil to protect the dye from bright light.

4. Gently agitate the gel at room temperature.

- The staining time is 10 to 60 minutes, depending on the thickness and percentage of the gel. For 1 mm-thick 15% poly-acrylamide gels, the signal is typically optimal at 40 to 60 minutes of staining.
- Once the optimal signal is achieved, additional staining time (several hours to overnight) does not enhance or degrade the signal. Gels can be left in stain for up to a week with only a small loss in sensitivity; our detection limits under these conditions are approximately 8–16 ng/band.

5. Rinse briefly with 7.5% acetic acid.

- This brief rinse (less than a minute) removes excess stain from the gel surface to reduce background fluorescence on the surface of the transilluminator or gel scanner.
- Molecular Dynamics has stated that 30 minutes of destaining in 7.5% acetic acid improves background and signal detection in their gel scanner. However, our own testing has shown that, for Polaroid 667® black-and-white photography, even a 10 minute destaining results in lower sensitivity.

Triton X-100 Gels

Triton® X-100 at 0.1% or greater will interfere with SYPRO dye staining. If Triton X-100 is used with your gel, we recommend soaking the gel in two to three changes of buffer to be sure the Triton X-100 is diluted out, and then incubating the gel in 0.05% SDS for 30 minutes before staining as usual.

2-D Gels and IEF Gels

SYPRO Orange and SYPRO Red protein gel stains are not suitable for staining proteins in IEF gels, and they show reduced sensitivity when staining proteins on 2-D gels. For these applications, we recommend SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900).

Nondenaturing Gels

Protein can be stained after native gel electrophoresis by dissolving the SYPRO dyes in water and then following the protocol above.

Staining proteins in nondenaturing gels is highly protein-selective and will generally be less sensitive than staining pro-

teins in SDS gels; however, because there is essentially no background fluorescence, photographic exposures can be very long.

If it is not necessary to maintain the protein in a native state after electrophoresis, the best sensitivity can be achieved if the gel is soaked in 0.05% SDS for about 30 minutes and then stained with a solution of the SYPRO dye diluted in 7.5% acetic acid.³

Staining During Electrophoresis

SYPRO Orange and SYPRO Red protein gel stains can be dissolved in the cathode (top) running buffer to stain proteins as the gel runs. The SYPRO stock solution can be diluted 500-fold into the cathode running buffer. The dye moves through the gel with the SDS front, so that all sizes of protein are stained. Staining does not influence relative migration of proteins through the gel. This method results in poorer protein staining than the standard post-staining method and requires the same amount of time as the gel must be destained for 15 to 40 minutes in 7.5% acetic acid to reduce background fluorescence.

The SYPRO Red and SYPRO Orange stains cannot be used to pre-stain protein samples for SDS gels. Loading solutions contain so much SDS that the dye simply localizes in the free SDS and binds very little to the proteins. For nondenaturing gels it might be possible to use SYPRO dyes as pre-stains, but we have not tried this. SYPRO Red stain has been used as a pre-stain for capillary electrophoresis.⁶

Viewing and Photographing the Gel

Gels may be left in staining solution overnight without losing sensitivity. However, the fixation in acetic acid is relatively mild, so for low percentage gels or very small proteins, photographs should be taken as soon as possible after staining, before the proteins begin to diffuse.

Viewing the Gel

Gels may be visualized on a standard 300 nm UV transilluminator or with a blue-light transilluminator like the Clare Chemical Dark Reader™. We recommend cleaning the surface of the transilluminator with water and a soft cloth after using to minimize the buildup of fluorescent dyes on the surface.

- Place the gel directly on the transilluminator. Plastic wraps, such as Saran® Wrap, fluoresce on their own and even more when exposed to SYPRO Orange or SYPRO Red stain. This gives a large background signal if the gel is sitting on a piece of plastic wrap on a UV transilluminator and makes it impossible to get good sensitivity.
- Pharmacia PhastGels® have polyester backing material (Gelbond®) which is not only highly autofluorescent, but also binds the SYPRO stains, producing additional background fluorescence. Consequently, the plastic backing should be removed before trying to visualize your results. Pharmacia markets a gel backing remover for use with their Phast Transfer™ system.
- For those using a laser-excited gel scanner, we recommend the SYPRO Orange stain for argon laser-based instruments and the SYPRO Red stain for instruments that employ green He-Ne or Nd:YAG lasers (Figure 2).

Photography with a Polaroid Camera

Photographing the gel is essential to obtain high sensitivity. The camera's integrating effect can make bands visible that are not visible to the eye. The highest sensitivity with a Polaroid® camera will be obtained using Polaroid 667 black-and-white print film and the SYPRO photographic filter (S-6656).⁷

- Standard ethidium bromide filters should not be used as they will block much of the light and lead to lower sensitivity. Supplemental UV blocking filters are not usually required.
- Polaroid 667 film is a fast film with an ISO rating of ASA 3000. The use of different film types may require longer exposure times or different filters.
- Exposure time will vary with the intensity of the illumination source: with an f-stop of 4.5, typically 2 to 5 seconds for SYPRO Orange stain and 3 to 8 seconds for SYPRO Red stain.
- We generally observe detection limits of ~50 ng protein/band with 300 nm transillumination and ~4–8 ng protein/band in a photograph taken with Polaroid 667 black-and-white print film. Our detection limits of 4–8 ng/band are obtained using a Fotodyne® Foto/UV® 450 Ultraviolet Transilluminator, which has six 15-watt bulbs that provide peak illumination at 312 nm. When using weaker illumination sources, exposures must be adjusted accordingly.
- Noticeable photobleaching can occur after several minutes of exposure to ultraviolet light. If a gel becomes photobleached, it can be restained by simply returning it to the staining solution.

Photography with a CCD Camera

CCD cameras also provide good sensitivity, however the SYPRO photographic filter may not be optimal. Contact the manufacturer of your camera system for the optimal filter sets to use.

Storing the Stained Gel

Gels may be stored by keeping them protected from light in the staining solution. Although the signal does decrease somewhat after several days, your gels may retain a usable signal for many weeks, depending on the amount of protein in your bands.

Gels may be dried between sheets of cellophane, although there is sometimes a slight decrease in sensitivity. Store the dried gel in the dark to prevent photobleaching.

- If the gels are dried onto paper, the light will scatter and the sensitivity will decrease.
- If the gel is dried between sheets of other plastic, the plastic typically used is not transparent to UV light.

Destaining the Gel

Gels may be mostly destained by incubation overnight in 0.1% Tween® 20. Alternatively, incubation in several changes of 7.5% acetic acid will eventually remove all of the stain. Incubation in methanol will strip off dye and SDS but will also precipitate proteins.

Tips

- The SDS front at the bottom of the gel stains very heavily with SYPRO Orange and SYPRO Red stains. Unless the proteins of interest are co-migrating with the SDS front, it will be advantageous to run the SDS front off the gel.
- Colored stains and marker dyes, as well as commercially pre-stained protein markers, interfere with SYPRO dye staining and quench fluorescence.
- Highly-colored prosthetic groups (e.g. heme) that remain bound in native gels will quench fluorescence of the SYPRO Orange and SYPRO Red stains.
- Odd marks on stained gels can be caused by several factors. If the gel is squeezed, a mark appears that stains heavily with the SYPRO dyes. This is probably a localized high concentration of SDS that has difficulty diffusing out. Glove powder can also give background markings, so we recommend rinsing or washing gloves prior to handling gels.
- Staining with the SYPRO Orange dye occasionally results in gels with scattered fluorescent speckles. We don't know what the speckles are and have not been able to completely get rid of them, but they seem to be only a cosmetic problem — they don't reduce the dye's sensitivity.
- SYPRO dye-stained gels can be restained with either Coomassie brilliant blue or with silver stain procedures. In fact, for some silver staining methods, we have found that prestaining with SYPRO dyes actually increases the rate of staining and the sensitivity for detection.
- To stain gels previously stained with Coomassie brilliant blue stain, the stain must be completely removed as it will quench the fluorescence of SYPRO dyes. Soaking the gel in either 30% methanol or 7.5% acetic acid with several changes of the destaining solution will be effective at removing the Coomassie stain. Once the Coomassie dye has been removed, the gel should be incubated in 0.05% SDS for 30 minutes before staining with the SYPRO stain as usual.

References

1. Anal Biochem 239, 223 (1996); 2. J NIH Res 7, 82 (1995); 3. Anal Biochem 239, 238 (1996); 4. Short Protocols in Molecular Biology, Second Edition, Ausubel et al, John Wiley & Sons (1992); 5. Nature 227, 680 (1970); 6. Electrophoresis 19, 2169 (1998); 7. Anal Biochem 248, 168 (1997).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
S-6650	SYPRO® Orange protein gel stain *5000X concentrate in DMSO*	500 µL
S-6651	SYPRO® Orange protein gel stain *5000X concentrate in DMSO* *special packaging*	10x50 µL
S-6656	SYPRO® photographic filter	each
S-12012	SYPRO® Protein Gel Stain Starter Kit	1 kit
S-6653	SYPRO® Red protein gel stain *5000X concentrate in DMSO*	500 µL
S-6654	SYPRO® Red protein gel stain *5000X concentrate in DMSO* *special packaging*	10x50 µL

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