*Revised: 13–January–2001*

# *SYPRO® Tangerine Protein Gel Stain (S-12010)*

**Quick Facts**

***Storage upon receipt:***
- Room temperature
- Desiccate
- Protect from light

***Ex/Em:*** 300, 490/640 nm

## *Introduction*

Molecular Probes' proprietary SYPRO® Tangerine protein gel stain is an extremely versatile stain for proteins in SDS gels. The staining procedure is fast, simple and sensitive and does not require the use of acids or organic solvents — staining can be performed in a simple saline solution. Proteins stained without fixation can be used for zymography (in-gel enzyme activity) assays, provided SDS does not inactivate the protein of interest (Figure 1). Stained proteins can also be eluted from gels and used for further analysis. The stain does not alter protein structure and so does not interfere with analysis by mass spectrometry. In addition, staining does not interfere with the transfer of proteins to blotting membranes, allowing visualization of proteins *before* proceeding with Western blotting or other blotting applications. If protein fixation is preferred, the dye also works in 7% acetic acid or in 12.5% trichloroacetic acid solutions.

Proteins stained with the SYPRO Tangerine dye can be easily visualized using a standard UV or blue-light transilluminator or using a laser scanner. SYPRO Tangerine stain used without harmful fixatives is ideal for classroom use, especially when visualized with a blue-light transilluminator, which minimizes exposure to UV light.

SYPRO Tangerine protein gel stain provides the following additional advantages over conventional colorimetric stains:

- **High sensitivity.** SYPRO Tangerine protein gel stain can detect 4 to 8 ng of protein per minigel band, making it far more sensitive than Coomassie® Brilliant Blue (CBB) staining and as sensitive as many silver staining techniques.
- **Rapid staining.** Staining is complete in less than an hour.
- **Simple staining procedure.** After electrophoresis, the gel is simply stained, rinsed and photographed — no fixation or destaining steps are required and there is no fear of overstaining the gel.
- **Compatibility with standard laboratory equipment.** Stained proteins can be visualized using a standard 300 nm UV transilluminator or a laser scanner (Figure 2).
- **Cost-effective staining.** SYPRO Tangerine staining is less expensive than silver staining and requires much less hands-on time.
- **Low protein-to-protein variability.** Because SYPRO Tangerine dye interacts with the SDS coat around proteins in the gel, it gives more consistent staining between different types of proteins compared to CBB or silver staining and never exhibits negative staining.
- **High selectivity for proteins.** SYPRO Tangerine protein gel stains detects just about any protein 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.** SYPRO Tangerine stain interacts primarily with the SDS-protein micelle during gel staining. Since SDS binds to protein with a fairly constant stoichiometry of 1.4 to 1, the fluorescence intensity of stained bands is linear with protein quantity over three orders of magnitude, a much broader range than either CBB or silver staining can provide.

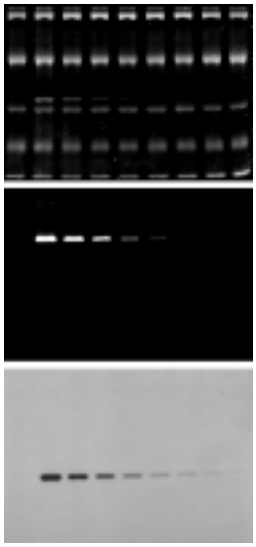

***Figure 1.*** *SDS polyacrylamide gels stained for total protein with SYPRO Tangerine protein gel stain and subsequently stained for specific enzymatic activities. Two identical gels were run with samples of protein molecular weight standards (leftmost lanes) and protein molecular weight standards mixed with decreasing amounts of Escherichia coli β-glucuronidase and rabbit-liver esterase. Both gels were first stained with SYPRO Tangerine protein gel stain (one gel shown, top) and then one was stained with ELF®-97 β-D-glucuronidase substrate (E-6587) for the detection of β-glucuronidase activity (middle) and the other with α-naphthyl acetate and Fast Blue BB for the detection of esterase activity (bottom).*

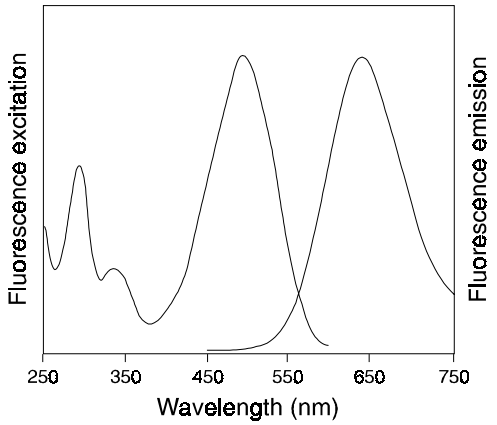


***Figure 2.*** *Emission and excitation spectra for the SYPRO Tangerine protein gel stain bound to protein.*

## Materials

### Contents

SYPRO Tangerine protein stain is provided in a 500 µL unit size, as a 5000X concentrated solution in dimethylsulfoxide (DMSO). One 500 µL unit size prepares a total of 2.5 L of working stain solution, which is sufficient to stain ~50 polyacrylamide mini-gels.

We also offer the SYPRO Tangerine stain as a component of the SYPRO Protein Gel Stain Starter Kit (S-12012), which includes:

- SYPRO Orange protein gel stain, 50 µL
- SYPRO Red protein gel stain, 50 µL
- SYPRO Tangerine protein gel stain, 50 µL
- SYPRO protein gel stain photographic filter

### Storage

The SYPRO Tangerine stock solution should be stored desiccated and protected from light at either room temperature, 4°C or -20°C. When stored properly, the stock solution is stable for at least 6 months. Staining reagent diluted in buffer can be stored protected from light at 4°C for at least 3 months.

### Handling

*Before opening, the vial should be allowed to warm to room temperature.* Water condensation on the wall of a cold vial, when mixed into the DMSO stock solution, can cause the dye to precipitate. After thawing completely, the vial should be 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

Although eliminating methanol and acetic acid from staining solutions makes the SYPRO Tangerine staining protocol environmentally friendly, no data are available on the toxicity of SYPRO Tangerine dye itself. For disposal, SYPRO Tangerine stain should be poured through activated charcoal or other compatible combustible material and then burned in a chemical incinerator to destroy the dye. All Federal, state and local environmental regulations should be observed when disposing of the dye.

## Staining Protocol

### Staining proteins after electrophoresis

**1. Prepare the staining solution by diluting the stock SYPRO reagent 1:5000 and mixing vigorously.**

- If the proteins are to be used for subsequent analysis, dilute the stock solution into 50 mM phosphate, 150 mM NaCl, pH 7.0. Alternatively, a wide range of buffers are compatible with the stain including: formate, pH 4.0; citrate, pH 4.5; acetate, pH 5.0; MES, pH 6.0; imidazole, pH 7.0; HEPES, pH 7.5; Tris acetate, pH 8.0; Tris-HCl, pH 8.5; Tris borate, 20 mM EDTA, pH 9.0; and bicarbonate, pH 10.0. Buffers should be prepared as 50–100 mM solutions containing 150 mM NaCl. The stock solution may also be diluted directly into 150 mM NaCl. If no fixative is used before or during staining, some diffusion of the protein bands may occur, especially for smaller proteins.
- If the proteins are to be transferred to a blot, dilute the SYPRO Tangerine stain stock solution into 50 mM phosphate, 150 mM NaCl, pH 7.0 to stain the gel. After staining, transfer the gel to Western blotting buffer containing 0.1% SDS. The SDS is not absolutely required, but it helps in the transfer of some proteins to the blot. Acetic acid and other fixatives will interfere with transfer of proteins to blotting membranes.
- For the best band morphology and to minimize diffusion of the proteins, dilute the SYPRO Tangerine stock solution in 7.5% (v/v) acetic acid to fix the proteins in the gel. For low percentage gels and for very small proteins, 10% acetic acid will result in better retention of the protein in the gel without compromising sensitivity. Do not fix the proteins in the gel using methanol-containing solutions. Methanol removes the SDS coat from proteins, strongly reducing the signal from SYPRO Tangerine stain.
- 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.

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

- For one or two standard-size minigels, use at least 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.

**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 gel thickness and percentage of polyacrylamide. For 1 mm–thick 15% polyacrylamide gels, the signal is typically optimal at 30 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 4–8 ng/band.

### 2-D Gels and IEF Gels

SYPRO Tangerine protein gel stain is not suitable for staining proteins in IEF gels and shows only moderate sensitivity when staining proteins on 2-D gels. For these applications, we recommend our SYPRO Ruby IEF protein gel stain (S-12003) and SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900), respectively.

## Viewing and Photographing the Gel

Gels may be left in staining solution overnight without loss of sensitivity. However, unfixed proteins will eventually diffuse out of the gel, especially for low percentage gels or very small proteins, so photographs should be taken as soon as possible after staining. The two excitation maxima — at ~300 nm and at ~490 nm — make it possible to visualize stained proteins using either a UV or a visible light source. The emission maximum is at ~640 nm (Figure 2).

## 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.
- Gels may also be visualized using laser scanners.
- Place the gel directly on the transilluminator or laser scanner. Plastic wraps, such as Saran® Wrap, fluoresce on their own and even more when exposed to SYPRO Tangerine 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 Tangerine stain, 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 PhastTransfer™ system.

### Photography and Archiving

Photography of the gel is essential to obtain high sensitivity. The integrating effect of a camera or laser imaging system can make bands visible that are not visible to the eye.

**Photography with a Polaroid Camera.**

- The highest sensitivity with a Polaroid® camera will be obtained using Polaroid 667 black-and-white print film and the SYPRO protein gel stain photographic filter (S-6656).
- The use of typical ethidium bromide filters 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, an exposure of 2–5 seconds is usually adequate.
- We generally observe detection limits of ~500 ng protein/band in room light, ~50 ng protein/band with 300 nm transillumination and ~1–2 ng protein/band in a photograph taken with Polaroid 667 black-and-white print film. Our detection limits of 1–2 ng/band were obtained using a Fotodyne® Foto/UV7® 450 Ultraviolet Transilluminator, which has six 15-watt bulbs that provide peak illumination at 312 nm. When using weaker illumination sources, exposures must be correspondingly longer.
- Although our detection limits are 1–2 ng/band for most proteins, we would like to emphasize that bands containing 5–10 ng/protein are readily detected and those containing less protein require longer exposures and sharp bands for good visualization. Longer exposures can result in higher background.
- 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.**

- If using a cooled CCD-camera, the best images are obtained by digitizing at about 1024 × 1024 picture elements (pixels) resolution with 12- or 16-bit gray scale levels assigned per pixel.
- 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 dried between sheets of cellophane (Bio-Rad), 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 gels

SYPRO Tangerine stain is readily destained by incubation in 7% acetic acid, 10% methanol.

## Tips

- The SDS front at the bottom of the gel stains very heavily with SYPRO Tangerine stain. Unless the proteins that interest you 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 prestained protein markers, may interefere with SYPRO Tangerine dye staining and quench fluorescence.
- Highly colored prosthetic groups (e.g. heme) that remain bound in native gels will quench fluorescence of the SYPRO Tangerine stain.
- 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 Tangerine dye. 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 Tangerine 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 Tangerine 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 Tangerine dye 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 Tangerine dye. 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.

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

| Cat # | Product Name | Unit Size |
|---|---|---|
| S-6656 | SYPRO® protein gel stain photographic filter | each |
| S-12012 | SYPRO® Protein Gel Stain Starter Kit | 1 kit |
| S-12010 | SYPRO® Tangerine protein gel stain *5000X concentrate in DMSO* | 500 µL |

## *Contact Information*

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information


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