

product code
RPN 5801/2/3/4

SYPRO Orange and Red protein gel stains

SYPRO™ Orange and Red protein gel stains enabling fast, simple and sensitive staining of proteins in electrophoretic gels.

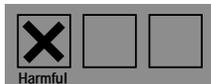
Warning

For research use only.

Not recommended or intended for diagnosis of disease in humans or animals.

Do not use internally or externally in humans or animals.

Contains DMSO. See safety data sheet supplied.



Components

RPN 5801
SYPRO Orange gel stain
5000x concentrate in
DMSO, 500µl

RPN 5802
SYPRO Orange gel stain
5000x concentrate in
DMSO, 10x50µl

RPN 5803
SYPRO Red gel stain
5000x concentrate in
DMSO, 500µl

RPN 5804
SYPRO Red gel stain
5000x concentrate in
DMSO, 10x50µl

Sufficient material in each to prepare 2.5 litres of working stain solution which is sufficient to stain ~50 polyacrylamide minigels.

Description

SYPRO Orange and Red protein gel stains are designed for fast, simple, sensitive staining of proteins in electrophoretic gels.

The staining properties of the two SYPRO dyes are similar, and both are equally suitable for use in most procedures. Our scientists have noted that the SYPRO Orange gel stain is slightly brighter, whereas the SYPRO Red gel stain has somewhat lower background fluorescence. 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). Both dyes are efficiently excited by UV or broadband illumination (Figure 2) and, with the correct filters, work well with CCD camera archiving systems.

SYPRO Orange and Red protein gel stains are not suitable for staining proteins on blotting membrane or in IEF gels and they show reduced sensitivity when staining proteins on 2-D gels.

Other materials required

(not supplied with these products)

- Standard gel electrophoresis equipment and solutions (eg from Hoefer™)
- UV transilluminator
- Small plastic box lids or sealable plastic bags

Handling

Packaging: Screw cap plastic vials contained within foil bag.

Storage: Store in the dark at room temperature, 2-8°C or -15°C to -30°C

Stored as above, stock solutions are stable for 6-12 months.

Diluted staining reagent (in buffer or acetic acid) is stable for 3 months when stored in sterile detergent free glass or plastic bottles at 2-8°C in the dark.

SYPRO Orange and Red protein gel stains provide the following advantages over conventional colorimetric stains:

- **High sensitivity.** Detection of 1-2ng of protein per minigel band – more sensitive than Coomassie™ Brilliant Blue and as sensitive as silver staining
- **Rapid.** Staining complete in <1 hour^(1,2)
- **Simple.** After electrophoresis, simply stain, rinse and photograph^(1,3)
- **Compatible.** Can be used with standard 300nm UV transilluminator or a laser scanner
- **Low protein – protein variability.** Because SYPRO Red and Orange dyes interact with the SDS coat around proteins in the gel they give more consistent staining between different types of protein⁽¹⁾ and never exhibit negative staining. They also stain glycosylated proteins well.
- **High selectivity for proteins.** They detect proteins down to ~6500Da without staining nucleic acid or lipopolysaccharide contaminants often present.
- **Broad linear range of detection.** The fluorescence intensity of SYPRO stained bands is linear with protein quality over three orders of magnitude.

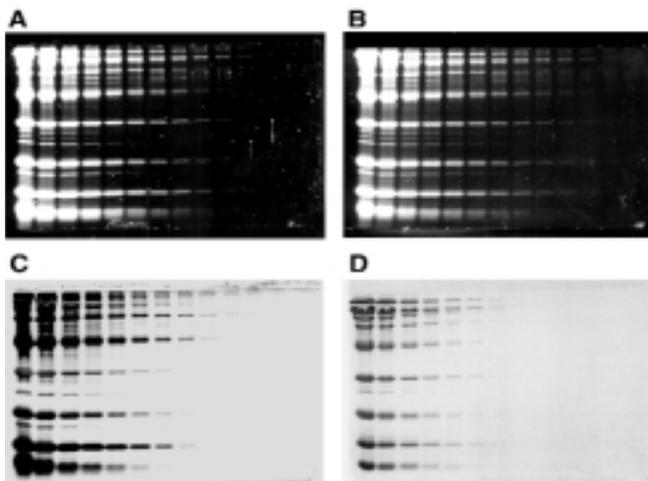


Figure 1. Identical polyacrylamide minigels stained with A) SYPRO Orange gel stain, B) SYPRO Red gel stain, C) silver stain and D) Coomassie Brilliant Blue (CBB) stain according to standard protocols. The SYPRO-stained gels were photographed using 300nm transillumination, a SYPRO Orange/Red protein gel stain photographic filter 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.

Critical parameters

The following points are critical to the performance of this protocol and should be strictly observed

- Store SYPRO gel stains protected from light.
- Allow vials to equilibrate to RT, sonicate to redissolve any dye particles and centrifuge briefly before opening.
- When running SDS-polyacrylamide gels use 0.05% SDS in the running buffer.
- Use the staining reagent at the dilution stated for optimum results.
- Do not fix the proteins in the gel with methanol as this will result in a reduced signal with SYPRO Red and Orange stains.

Safety warnings and precautions

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Warning: Contains DMSO. See safety data sheet supplied.

The toxicity of the SYPRO protein gel stains has not been fully evaluated and no data is currently available. Please handle with care.

Waste 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 1g of activated charcoal binds at least 98% of the SYPRO Orange or SYPRO Red dye present in 2.5l of 1x staining solution prepared in 7.5% acetic acid, which is equivalent to the amount of dye in 500µl of the 5000x concentrated DMSO solution.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Protocol

1

SDS-polyacrylamide gel electrophoresis

Prepare fresh running buffer and run SDS-polyacrylamide gel according to standard protocols,^(4,5) with the following exception. We recommend using 0.05% SDS in the running buffer (instead of the usual 0.1% SDS). This has been shown to be the optimal concentration of SDS for sensitivity and speed. Higher concentrations require extended washing to reduce background fluorescence and lower concentrations of SDS (or old running buffer) result in poor resolution of bands.

2

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, sensitivity is greatly reduced after the second use.
- 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 Red stain in standard transfer buffer. However, as this results in lower sensitivity staining we recommend staining with SYPRO Tangerine, RPN 5805 for blotting techniques.
- 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 Red stains.

2. Pour the staining solution into a small plastic dish

- For one or two standard-size minigels, use about 50ml of staining solution. For larger gels, use between 500 and 750ml of staining solution.
- Clean and rinse the staining dishes well before use as detergent will interfere with staining.
- Gels may also be stained in sealable plastic bags, ensuring the correct volume of staining solution is used.

3. Place the gel into the staining solution

- Cover the container with aluminium 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 or percentage of the gel. For 1mm thick 15% polyacrylamide gels, optimal signal is achieved after 40 to 60 minutes staining.
- 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 2-4ng/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.
- 30 minutes of destaining in 7.5% acetic acid has been shown to improve background and signal detection in a gel scanner. However, testing has shown that, for Polaroid™ 667 black-and-white photography, even a 10 minute destaining results in lower sensitivity.

Staining proteins during electrophoresis

- SYPRO 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 5000-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. However this results in poorer protein staining and higher background fluorescence.

- SYPRO Red and Orange stains cannot be used as prestains for SDS gels due to their affinity for the SDS.

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 Red protein gel stains are not suitable for staining proteins on IEF gels, and they show reduced sensitivity when staining proteins on 2-D gels.

Nondenaturing Gels

Protein can be stained after native gel electrophoresis by dissolving 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 proteins 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 SYPRO dye diluted in 7.5% acetic acid.⁽³⁾

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Viewing 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.

- Gels may be visualized on a standard 300nm UV transilluminator (eg from Hoefer). We recommend cleaning the surface of the transilluminator with water and a soft cloth after using to minimize the build up of fluorescent dyes on the surface.
- Place the gel directly on the transilluminator. Plastic wraps, such as SaranWrap™, fluoresce on their own and even more when exposed to SYPRO Orange or 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.
- Amersham Biosciences PhastGel™ has 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. Amersham Biosciences markets a gel backing remover for use with the PhastTransfer™ 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).

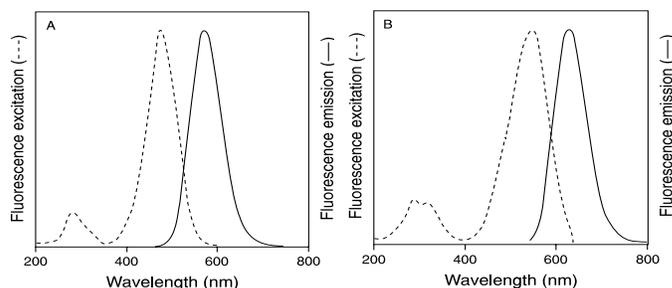


Figure 2. The fluorescence excitation and emission spectra of A) SYPRO Orange and B) SYPRO Red protein gel stains diluted 1:10,000 in water containing 0.05% SDS and 150µg/mL bovine serum albumin.

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Photographing the gel

Photography of the gel is essential to obtain high sensitivity. The camera's integrating effect 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 RPN5810⁽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 ASA3000. 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 2-5 seconds for SYPRO Orange stain and 3-8 seconds for SYPRO Red stain.
- We generally observe detection limits of ~50ng protein/band with 300nm transillumination and ~1-2ng/band in a photograph taken with a Polaroid 667 black and white print film. Our detection limits of 1-2ng/band are obtained using an Ultraviolet Transilluminator, which has six 15-watt bulbs that provide peak illumination at 312nm. When using weaker illumination sources, exposures must be correspondingly longer.
- Although our detection limits are 1-2ng/band for most proteins, we would like to emphasize that bands containing 5-10ng/ protein are more readily detected. Bands containing less than 5-10ng 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

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. The signal does decrease somewhat after several days, but, depending on the amount of protein in your bands, your gels may retain a usable signal for many weeks.

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 on to 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.

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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.

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Tips

- The SDS front at the bottom of the gel stains very heavily with SYPRO 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.
- Coloured stains and marker dyes, as well as commercially prestained protein markers, interfere with SYPRO dye staining and quench fluorescence.
- Highly-coloured prosthetic groups (e.g. heme) that remain bound in native gels will quench fluorescence of the SYPRO Orange and 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. However, they do not 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, p.223, 1996.
2. *J.NIH Res.*, 7, p.82, 1995.
3. *Anal. Biochem.*, 239, p.238, 1996.
4. *Short Protocols in Molecular Biology*, second edition, Ausubel et al., John Wiley & Sons, 1992.
5. *Nature*, 227, p680, 1970.
6. *Electrophoresis*, 19, p.2169, 1998.
7. *Anal. Biochem.*, 248, p.168, 1997.

Product information

| Product name | code |
|--------------------------------|----------|
| SYPRO Orange protein gel stain | |
| 500µl | RPN 5801 |
| 10x50µl | RPN 5802 |
| SYPRO Red protein gel stain | |
| 500µl | RPN 5803 |
| 100x50µl | RPN 5804 |

| Related products | |
|---|----------|
| SYPRO Tangerine protein gel stain | RPN 5805 |
| SYPRO protein gel stain starter kit | RPN 5811 |
| Protein molecular weight markers | |
| Broad range MW 6500-205000 | RPN 5800 |
| SYPRO protein gel stain photographic filter | RPN 5810 |

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