product code

RPN 5805

SYPRO Tangerine protein gel stains

SYPRO™ Tangerine protein gel stain enabling fast, simple and sensitive staining of proteins in electrophoretic gels.

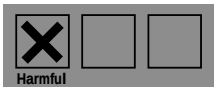
Warning

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 5805
SYPRO Tangerine gel stain 5000x concentrate in DMSO, 500µl

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

Description

SYPRO Tangerine protein gel stain is designed for fast, simple, sensitive staining of proteins in electrophoretic gels, without the use of organic solvents or acids. Staining can be performed in 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% trichloracetic acid solutions.

Proteins stained with 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 advantages over conventional colorimetric stains:

- **High sensitivity.** Detection of 4-8ng 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.** Beause SYPRO Tangerine dye interacts with the SDS coat around proteins in the gel it gives more consistent staining between different types of protein[1] and never exhibits negative staining.
- **High selectivity for proteins.** It detects proteins down to ~6500Da without staining nucleic acid or lipopoly-saccharide 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.

Other materials required

(not supplied with this product)

- Standard gel electro-phoresis 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 desiccated in the dark at room temperature, 2-8°C or -15°C to -30°C

Stored as above, stock solutions are stable for at least 6 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.

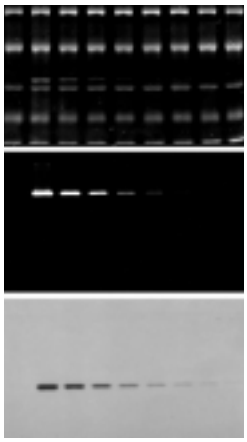


Figure 1. SDS polycrylamide gels stained for total protein with SYPRO Tangerine protein gel stain and subsequently stained for specific enzymatic activities. Two identical gels were run with samples of protein molecular weight standards (leftmost lanes) and protein molecular weight standards mix with 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).

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 Tangerine stain.

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.

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 care of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Protocol

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Staining proteins in the gel

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 50mM phosphate, 150mM NaCl, pH7.0. Alternatively, a wide range of buffers are compatible with stain including: formate, pH4.0; citrate, pH4.5; acetate, pH5.0; MES, pH6.0; imidazole, pH7.0; HEPES, pH7.5; Tris acetate, pH8.0; Tris-HCL, pH8.5; Tris borate, 20mM EDTA, pH9.0; and bicarbonate, pH10.0. Buffers should be prepared as 50-100mM solutions containing 150mM NaCl. The stock solution may also be diluted directly into 150mM 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 50mM phosphate, 150mM NaCl, pH7.0 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 strain.
- 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 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.

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 30 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 4-8ng/band.

2-D Gels and IEF Gels

SYPRO Tangerine protein gel stains is not suitable for staining proteins on IEF gels, and shows only moderate sensitivity when staining proteins on 2-D gels.

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Viewing the Gel

Gels may be left in staining solution overnight without losing sensitivity. However, unfixed proteins will eventually diffuse out of the gel, especially low percentage gels or very small proteins. Photographs should be taken as soon as possible after staining, before the proteins begin to diffuse.

The two excitation maxima, at ~300nm and at ~490nm, make it possible to visualize stained proteins using either UV or a visable light source. The emission maxima is at ~640nm (Figure 2).

- 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.

- Gels may also be visualized using laser scanners.
- Place the gel directly on the transilluminator or laser scanner. Plastic wraps, such as SaranWrap™, 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.
- 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.

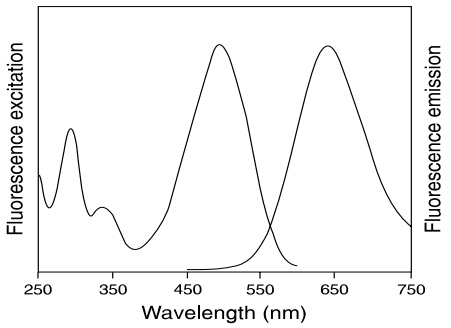

Figure 2. Emission and exciation spectra for the SYPRO Tangerine protein gel stain bound to protein.

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Photographing the gel

Photography of the gel is essential to obtain high sensitivity. The integrating effect of a camera or laser scanning 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 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 is usually adequate.
- We generally observe detection limits of ~500ng protein/band in room light, ~50ng protein/band with 300nm transillumination and ~1–2ng/band in a photograph taken with a Polaroid 667 black and white print film.
- 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

- If using a cooled CCD-camera, the best images are obtained by digitizing at about 1024 x 1024 picture elements (pixels) resolution with 12- or 16-bit grey 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, 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

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

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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 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 occasionlly results in gels with scattered fluorescent speckles. However, they do not 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.

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 Tangerine protein gel stain	RPN 5805

Related products	
SYPRO Orange protein gel stain	
500µl	RPN 5801
10x50µl	RPN 5802
SYPRO Red protein gel stain	
500µl	RPN 5803
100x50µl	RPN 5804
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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