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SYPRO® Red & Tangerine Protein Gel Stains

Introduction

SYPRO® Protein Gel Stains are highly sensitive fluorescent stains for the rapid detection of proteins in polyacrylamide gels. The detection sensitivity **exceeds** Coomassie® brilliant blue, approaches that of silver stain and can detect as little as 1 ng – 8 ng of protein per band. Simply soak the gel in a solution of 1X SYPRO® Stain and see results within 40 – 60 minutes without destaining. These stains do not require a separate fixation step. Gels stained with SYPRO® Protein Gel Stains exhibit low background and minimal protein-to-protein staining variability. Gels can be documented using standard imaging systems.

SYPRO® STAIN	Routine high-performance staining	Mass Spectrometry	Argon laser-based gel scanners	Green He-Ne or Nd:YAG lasers	Zymography	Staining gel before Western transfer
Red	•	•		•		
Tangerine	•	•	•		•	•

Contents

500 µl of the 5,000X stock solution is enough to prepare 2.5 liters of working stain solution.

Storage and Handling

- SYPRO® Stains are supplied as 5,000X concentrated stock solutions in DMSO. Store dessicated at room temperature protected from light. When stored per directions the stock solutions are stable for 6 to 12 months from the date of receipt.
- Diluted stock solutions are stable for at least three months when protected from light and stored at 4°C in a clean, detergent-free glass or plastic bottle.

Cautions

The DMSO stock solutions should be handled with caution as DMSO is known to facilitate the entry of organic molecules into tissues. **We strongly recommend using double gloves when handling the DMSO stock solutions.** Please refer to the MSDS for more details. No data are available on the toxicity of the SYPRO® Protein Gel Stains.

Disposal

Stain solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal.

Tips for Staining

- **For gels to be transferred, use SYPRO® Tangerine Protein Gel Stain.**
- **Before you stain -** Run SDS-polyacrylamide gels according to standard protocols. Prepare and run gels using 0.05% SDS. Gels with 0.1% SDS may require a slightly longer staining time or a 10 minute rinse in water to reduce the background.
- **Do not fix the proteins in the gel using methanol-containing solutions**. Methanol removes the SDS coat from the proteins, strongly reducing the detection signal from the stains.
- **Staining the gel in transfer buffer will result in decreased sensitivity.** For blotting techniques, we recommend staining the gel with SYPRO® Tangerine Protein Gel Stain which does not require an acetic acid fixation step. Dilute SYPRO® Tangerine Gel Stain in 50 mM phosphate, 150 mM NaCl, pH 7.0.
- The SDS front at the bottom of the gel stains heavily with SYPRO® Red and Tangerine Stains. Unless the protein of interest co-migrates with the SDS front we recommend running the SDS front off the end of the gel.
- Colored stains such as Coomassie® blue stains and colored protein markers may interfere with SYPRO® staining and quench fluorescence. To stain gels previously stained with Coomassie® blue stain, soak the gel in several changes of 7.5% acetic acid to remove the Coomassie stain. Then incubate the gel in 0.05% SDS for 30 minutes and stain with SYPRO® Stain as usual.
- Glove powder can leave background markings on gels. Rinse or wash gloves prior to handling gels.
- Clean the surface of the transilluminator after each use with deionized water and a soft cloth. Fluorescent stains (such as SYPRO® Stains or ethidium bromide) can accumulate on the transilluminator and may cause high background.
- Handle gels carefully to avoid non-specific staining of areas of the gel that have been squeezed.
- SYPRO® Stains may be photobleached after several minutes of exposure to UV light. If a gel becomes photobleached, restain by incubating in the staining solution.

- SYPRO® stained gels can be restained with Coomassie® blue or silver stain procedures.
- Plastic wraps and GelBond® Film will autofluoresce when exposed to UV light resulting in very high background. For imaging backed gels, we recommend the use of Clare Chemical's Dark Reader® transilluminator.

General Procedure for Staining Proteins with SYPRO® Protein Gel Stains

1. Run SDS-polyacrylamide gels according to standard protocols.
   NOTE: To reduce background staining with SYPRO® Red Stain use 0.05% SDS in the running buffer. Gels run in 0.05% SDS show no change in the migration pattern of proteins.
2. Dilute the 5,000X concentrate to a 1X solution, in 7.5% (v/v) acetic acid, in a clear plastic polypropylene container. For example, for every 10 ml acetic acid add 2 µl stock SYPRO® Stain solution.
3. Mix well.
4. Place the gel into the staining container and cover with a lid to protect from light.
5. Gently agitate the gel at room temperature.
6. Stain the gel for 40-60 minutes.
   NOTE: The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.
7. Briefly rinse the gel in 7.5% acetic acid.
8. Remove the gel from the staining container and photograph the gel following the procedures outlined below.

Staining Nondenaturing Gels with SYPRO® Red Protein Gel Stain

There are 2 options for staining proteins with SYPRO® Red Stain after native gel electrophoresis.

Option 1

Dissolve the stain 1:5,000 in distilled water and follow the general protocol for staining proteins with SYPRO® Protein Gel Stains.
NOTE: This will be highly protein-selective, and will be less sensitive than staining proteins in SDS gels. To increase the signal, a long film exposure can be used since the background fluorescence is essentially zero.

Option 2

Soak the gel after electrophoresis in 0.05% SDS for 30 minutes, then stain with a 1:5,000 solution of SYPRO® Stain diluted in 7.5% acetic acid.
NOTE: Proteins will be denatured and fixed after electrophoresis using this treatment.

Staining Proteins with SYPRO® Tangerine Protein Gel Stain: Non-fixing Protocol

SYPRO® Tangerine Protein Gel Stain is an extremely versatile fluorescent stain that does not alter protein structure or interfere with the transfer of proteins to blotting membranes. The stain can be diluted into a wide range of buffers with a pH range from 4-10 and if fixing is necessary, it can be diluted into 7.5% acetic acid following the General Protocol for Staining Proteins with SYPRO® Gel Stains. SYPRO® Tangerine Protein Gel Stain is compatible with the following buffers:

Formate pH 4.0	HEPES pH 7.5
Citrate pH 4.5	Tris acetate pH 8.0
Acetate pH 5.0	Tris-HCl pH 8.5
MES pH 6.0	Tris borate, 20 mM EDTA pH 9.0
Imidazole pH 7.0	Bicarbonate, pH 10.0

If proteins are to be used for subsequent analysis dilute SYPRO® Tangerine stain stock solution into 50 mM phosphate, 150 mM NaCl, pH 7.0 or use one of the buffers listed above prepared as 50 mM – 100 mM solutions containing 150 mM NaCl.

Staining Proteins for Subsequent Analysis such as Zymography or Western Blotting

Stained proteins can also be eluted from gels and used for further analysis such as mass spectrometry.
NOTE: If the gel will be blotted after staining with SYPRO® Tangerine Stain, stain the gel according to the procedure below, using an appropriate diluent (previously described). Add 0.1% SDS to the Transfer Buffer. This may help in the transfer of some proteins to the membrane.

1. Run SDS-polyacrylamide gels according to standard protocols.
   NOTE: It is not necessary to decrease the amount of SDS present in the running buffer when using SYPRO® Tangerine Gel Stain.
2. Dilute the 5,000X concentrate to a 1X solution of diluent in a clear plastic polypropylene container. For example, for every 10 ml diluent add 2 µl stock SYPRO® Stain solution.
3. Mix well.
4. Place the gel into the staining container and cover to protect from light.
5. Gently agitate the gel at room temperature.
6. Stain the gel for 40-60 minutes.
   NOTE: The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.
7. Remove the gel from the staining container and immediately photograph the gel following the procedures outlined below.

Viewing Gels Stained with SYPRO® Protein Gel Stains

All SYPRO® Protein Gel Stains have two excitation wavelengths; in the UV region at approximately 300 nm and the visible region between 470 and 610 nm. This makes it possible to visualize the stains using a UV transilluminator, the Dark Reader® transilluminator from Clare Chemical, CCD camera systems or laser scanners.

SYPRO® Stain	Emission	Excitation (nm)
Red	630	550, 300
Tangerine	640	490, 300

Photographing Gels Stained with SYPRO® Protein Gel Stains

- Protein bands stained with SYPRO® Protein Gel Stains are best seen by photographing the gel. The integrating effect of a camera or imaging system can detect bands that are not visible to the eye.
- Photobleaching can occur after extended (several minutes) exposure to UV light. If bleached, the gel can be restained with only a small decrease in sensitivity.
- We recommend SYPRO® Red Stain for green HE-Ne or Nd:YAG laser.
- The highest sensitivity using a Polaroid® camera can be obtained using Polaroid 667 black-and–white print film and the SYPRO® Protein Gel Stain Photographic Filter.
- Exposure time will vary with the intensity of the illumination source. Begin with an f-stop of 4.5 and an exposure of 2 - 8 seconds. Use of an ethidium bromide filter is not recommended as it blocks much of the light and leads to lower detection sensitivity.
- The SYPRO® Photographic Filter does not work with CCD camera systems. For CCD cameras use the emission and excitation data above and check with the camera manufacturer for the appropriate filter.

Staining Gels for Western Blot Transfer

SYPRO® Stains can be diluted in Western blot transfer buffer to stain gels that will subsequently be transferred. The stains do not interfere with antibody probing of proteins on the membrane or microsequencing as they are removed from the proteins during the Western blot procedure. Staining in transfer buffer is about 2- to 4-fold less sensitive versus staining in acetic acid. Proteins are not commonly visible on membrane after transfer. For staining when optimal protein activity is desired, use SYPRO® Tangerine Protein Gel Stain.

For SYPRO® Red Gel Stain, use a transfer buffer containing 25 mM Tris, 187 mM glycine and 20% methanol. Higher methanol concentrations, or SDS in the transfer buffer will result in reduced protein detection. **For SYPRO® Tangerine Gel Stain** dilute the stock solution in 50 mM phosphate, 150 mM NaCl, pH 7.0.

1. Briefly centrifuge the vial containing the stain to deposit the solution at the bottom of the vial.
2. Dilute an appropriate volume of the 5,000X stock, (2 µl/10 ml) in the appropriate diluent (previously described).
3. Mix vigorously.
4. Stain the gel for 40 to 60 minutes as described in the general staining protocol.
5. Follow the instructions for “Photography”.
6. Transfer the proteins to a membrane using standard protocols and fresh transfer buffer.

Destaining Procedures

Overnight incubation in 0.1% Tween® 20 will remove most of the stain. Soaking in several changes of 7.5% acetic acid will remove all of the stain. Soaking in methanol will strip off the dye, the SDS, and will also precipitate the proteins.

Restaining Procedures

Gels stained with SYPRO® Protein Gel Stains can be restained with either Coomassie® brilliant blue or with silver stain. Follow your standard protocol.

Gels stained with Coomassie® brilliant blue can be restained with SYPRO® Stains. To stain gels previously stained with Coomassie® brilliant blue stain, you must remove the Coomassie® brilliant blue as it will quench the SYPRO® Stains. Soak the gel in either 30% methanol or 7.5% acetic acid with several changes of the destaining solution. Incubate the gel in 0.05% SDS for 30 minutes. Stain with SYPRO® Stain following the general protocol.

SYPRO® Stain Troubleshooting Guide

Symptom	Causes	Solution/Explanation
No Bands seen on gel	Wrong UV light box	Use a UV light box with 300 nm-312 nm lamps. Be sure the lamps are producing appropriate intensity.
	Very little protein	At the lower end of SYPRO® Stain's sensitivity range, the protein bands may not be visible by eye, but will show in the photograph.
	Incorrect stain dilution	Diluting the stain below the recommended concentration will result in reduced staining sensitivity.
	Use of colored stains, marker dyes or prestained protein markers	Colored stains or marker dyes, as well as commercially prestained protein markers, may interfere with SYPRO® Red Stain and quench fluorescence. Use unstained markers.
	Stain container	Clean and rinse the staining dishes well before use as the detergent will interfere with staining. We recommend pipette-tip lid boxes, Rubbermaid® Servin'Saver® or Seal-A-Meal® type bags.
	Old running buffer	SDS can precipitate out of the running buffer, decreasing the stain's ability to bind. Use either fresh running buffer, or add SDS concentrate to buffer just prior to use.
	Destaining the gel	SYPRO® Stains do not require destaining. If required refer to destaining procedures.
No bands in photograph	High background.	Use the correct stain dilution. A higher staining concentration can result in increased background and quench fluorescence.
	High background	The high background can sometimes obscure the protein bands. Use of the proper filters during photography will decrease background, allowing the bands to be visible. Decreasing the gel's SDS concentration to 0.05% from 0.1% may also help reduce background.
	Wrong exposure	Vary exposure until the background is low and the protein bands are visible. See protocol for recommended exposures.
	Improper photographic filters	Use the recommended photographic filters. For systems that use Wratten® Filters, either the SYPRO® Filter or a Wratten® #9 works best. Use a Tiffen® #15 (yellow-15) Filter for systems that require threaded glass filters. For CCD cameras, consult the manufacturer for the appropriate filter (see protocol for excitation and emission maxima).
Bands visible on light box, but fade with time	Photobleaching of SYPRO® Stains	Restain the gel in fresh stain in the appropriate staining buffer.
No bands visible after staining in transfer buffer	Wrong methanol concentration	Use recommended methanol concentration in transfer buffer/stain. High methanol concentrations can strip SDS from the proteins, resulting in low detection levels.
No SYPRO® stained bands visible on Western blot after transfer	Proteins with low hydrophobicity	Only highly hydrophobic proteins will retain enough SYPRO® Stain to be visible on a membrane. SDS is stripped off proteins during transfer, resulting in very little retention of the SYPRO® Stain on most proteins.
Large blob of stain at bottom of gel	Binding of SYPRO® Stains to SDS front.	Run gel longer, or if running small proteins, change to Tris-Tricine buffer system.
Proteins not visible on native gels	SYPRO® Stains require SDS/protein complexes to bind to proteins	Refer to procedure for staining nondenaturing proteins.
Protein not visible on Phast™ System gels or gels backed with GelBond® PAG Film	SYPRO® Stain binding to GelBond® Film, which strongly autofluoresces	Remove GelBond® Film from the gel prior to staining. Use Clare Chemical's Dark Reader® transilluminator to image gels.

Ordering Information

Catalog No.	Description	Quantity
50542	SYPRO® Red Stain	10 x 50 µl
50543	SYPRO® Red Stain	500 µl
50556	SYPRO® Tangerine Stain	500 µl
50540	SYPRO® Photographic Filter	Each

Related Products

PAGEr® Precast Gels
ProSieve® Protein Marker
ProSieve® 50 Gel solution

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