Coomassie Fluor™ Orange Protein Gel Stain

C-33250 Coomassie Fluor™ Orange protein gel stain, 1 L
C-33251 Coomassie Fluor™ Orange protein gel stain, 5 L

Quick Facts

Storage upon receipt:

- Room temperature
- Protect from light

Ex/Em: 300 and 470/570 nm

Introduction

Molecular Probes' proprietary Coomassie Fluor™ Orange protein gel stain provides fast, simple, sensitive staining of proteins in electrophoretic gels and offers the following advantages over conventional colorimetric stains:

- **High sensitivity.** Detects as little as 8 ng of protein per minigel band, sensitivity as good as or better than Coomassie® brilliant blue (CBB) stains.
- **Rapid staining.** Staining is complete in less than an hour.
- **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 risk of overstaining the gel.
- **Compatibility with standard laboratory equipment.** Stained proteins can be visualized using a standard 300 nm UV transilluminator or a laser-based scanner.
- **Low protein-to-protein variability.** Because Coomassie Fluor Orange dye interacts with the SDS coat around proteins in the gel, it gives more consistent staining between different types of proteins compared to CBB staining and never exhibits negative staining. Coomassie Fluor Orange dye also stains glycoproteins.
- **High selectivity for proteins.** Coomassie Fluor Orange protein gel stain detects 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 Coomassie Fluor Orange dye–stained bands is linear with protein quantity over at least two orders of magnitude, providing accurate quantitation.

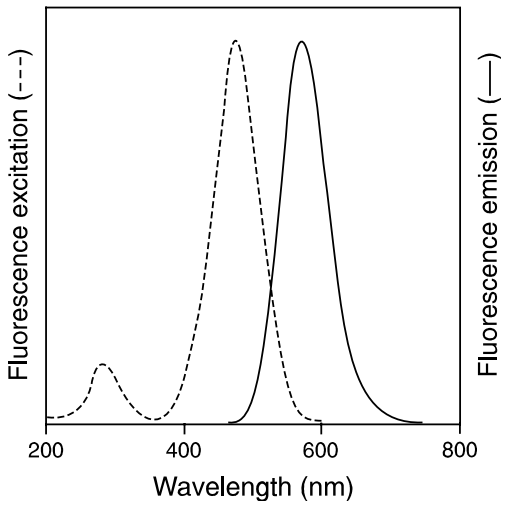

Figure 1. The fluorescence excitation and emission spectra of Coomassie Fluor Orange dye solution containing 0.05% SDS and 150 µg/mL bovine serum albumin.

Coomassie Fluor Orange protein gel stain is not recommended for staining proteins in 2-D, IEF or nondenaturing gels; for these applications, we recommend our SYPRO® Ruby protein gel stain (S-12000, S-12001, S-21900). Furthermore, Coomassie Fluor Orange is not suitable for staining proteins on blotting membranes; for this applications, we recommend SYPRO Ruby protein blot stain (S-11791).

Materials

Contents

Coomassie Fluor Orange protein gel stain is provided as a ready-to-use solution and is available as a 1 liter (C-33250) or 5 liter (C-33251) unit size. The 1 liter size provides sufficient reagent to stain ~20 polyacrylamide minigels, and the 5 liter size provides sufficient reagent to stain ~100 polyacrylamide minigels.

Storage

The Coomassie Fluor Orange solution should be stored at room temperature and protected from light. When stored properly, these products are stable for at least six months.

SDS-Polyacrylamide Gel Electrophoresis

Prepare and run SDS-polyacrylamide gel according to standard protocols. 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

1. 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.
- Diluting the stain below the recommended concentration will result in reduced staining sensitivity.
- The stain can be reused; however, we see dramatically reduced sensitivity, especially after the second reuse and therefore recommend using fresh staining solution for optimal sensitivity.
- Acetic acid is used in this stain. Acetic acid will interfere with transfer of the proteins to a blot. 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).
- Do not fix the proteins in the gel with methanol-containing solutions. Methanol removes the SDS coat from proteins, strongly reducing the signal from the Coomassie Fluor Orange protein gel stain.

2. Place the gel into the staining solution and gently agitate the gel at room temperature.

- Cover the container with aluminum foil to protect the dye from bright light.
- The staining time required is 30 to 60 minutes, depending on the thickness and percentage of the gel. For 1 mm–thick 15% polyacrylamide 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.

3. Rinse the gel 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.
- Do not destain for an extended period of time. Even 10 minutes of destaining results in lower sensitivity.

4. Wash once for 5 minutes with water (optional). This optional water wash may decrease some of the background, allowing visualization of low-abundance protein bands. The water wash may be repeated one time with no loss in sensitivity.

Triton X-100 Gels

Triton® X-100 at 0.1% or greater will interfere with Coomassie Fluor Orange staining. If Triton X-100 is used with the gel, soak the gel in two to three changes of buffer to be sure the Triton X-100 is diluted out, and then incubate the gel in 0.05% SDS for 30 minutes before staining as usual.

2-D Gels, IEF and Nondenaturing Gels

Coomassie Fluor Orange protein gel stain is not recommended for staining proteins in 2-D, IEF and nondenaturing gels. For these applications, we recommend SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900).

Viewing and Photographing the Gel

Gels may be left in the staining solution overnight without losing sensitivity. However, fixation in the Coomassie Fluor Orange dye solution 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

Stained gels may be visualized using one of the following excitation sources:

- a standard 300 nm UV transilluminator
- a blue-light transilluminator such as the Dark Reader™ (Clare Chemical Research)
- a laser or nonlaser visible-light gel scanner

For use with a visible-light scanner, choose the excitation light source and filters that most closely match the maximal excitation and emission wavelengths of Coomassie Fluor Orange dye (470/570 nm).

For optimal performance with all instruments,

- Clean the gel bed surface with water and a soft cloth after using to minimize the buildup of fluorescent dyes on the surface.
- Place the gel directly on the gel bed. Plastic wraps, such as Saran® Wrap, fluoresce on their own, and even more so when exposed to Coomassie Fluor Orange stain. Therefore, there will be high background signal if the gel is sitting on a piece of plastic wrap on a UV transilluminator, and it will be impossible to get good sensitivity.
- PhastGels® (Amersham Biosciences) have polyester backing material (Gelbond®), which is not only highly autofluorescent, but also binds the Coomassie Fluor Orange stain, producing additional background fluorescence. Consequently, the plastic backing should be removed before trying to visualize the gel.

Documentation of the Gel Image

The gel image can be easily documented using Polaroid photography, an image documentation system or a laser-based scanner. Regardless of which instrument is used for visualization, documentation of the gel through photography is *essential* to obtain high sensitivity. The camera's integrating effect can make bands visible that are not visible to the eye.

Polaroid Camera. 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).

- 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 is optimal for Coomassie Fluor Orange stain.
- We generally observe detection limits of ~4–8 ng protein/band with 300 nm transillumination in photographs taken with Polaroid 667 black-and-white print film. These detection limits 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.

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.

Scanning Instruments. For use with visible-excitation–light scanners, choose the excitation light source and filters that most closely match the maximal excitation and emission wavelengths of Coomassie Fluor Orange dye (470/570 nm).

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, the gels may retain a usable signal for many weeks, depending on the amount of protein in the 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 the dye and SDS but will also precipitate proteins.

Tips

- The SDS front at the bottom of the gel stains very heavily with the Coomassie Fluor Orange stain. 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, interefere with Coomassie Fluor Orange dye staining and quench the fluorescence.
- Highly colored prosthetic groups (e.g., heme) that remain bound in native gels will quench the fluorescence of the Coomassie Fluor Orange 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 Coomassie Fluor Orange 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 Coomassie Fluor 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.
- Coomassie Fluor Orange dye–stained gels can be restained with either Coomassie brilliant blue or with silver stain procedures.

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

Cat #	Product Name	Unit Size
C-33250	Coomassie Fluor™ Orange protein gel stain *ready-to-use solution*	1 L
C-33251	Coomassie Fluor™ Orange protein gel stain *ready-to-use solution* *bulk packaging*	5 L
S-6656	SYPRO® photographic filter	each

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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