Multiplexed Proteomics™ Transmembrane Protein Gel Stain Kit with Pro-Q® Amber and SYPRO® Ruby gel stains (M-33308)

Quick Facts

Storage upon receipt:

Protein Gel Stains (Components A and B)
- Room temperature
- Protect from light

Transmembrane Protein Standards (Component C)
- ≤–20°C

Ex/Em:

- 470/570 nm for Pro-Q Amber transmembrane protein gel stain
- 280, 450/610 nm for SYPRO Ruby protein gel stain

Introduction:

The Multiplexed Proteomics™ Transmembrane Protein Gel Stain Kit provides two different fluorescent gel stains from Molecular Probes — the Pro-Q® Amber stain, which is selective for transmembrane proteins in gels, and the SYPRO® Ruby stain, which stains all proteins. Thus, after sequentially staining and imaging a gel, transmembrane proteins can be discriminated from nontransmembrane proteins. For convenience, a mixture of bacteriorhodopsin (with seven transmembrane domains) and carbonic anhydrase (a nontransmembrane protein) is included with the kit to serve as a control. With the Pro-Q Amber stain, as little as 10 ng of bacteriorhodopsin can be detected, the staining intensity is linear over more than two orders of magnitude, and the staining intensity of bacteriorhodopsin is ≥20X greater than for that of the nontransmembrane protein (Figure 1). The Pro-Q Amber stain has been tested with a number of proteins, including those known to have hydrophobic transmembrane α-helices and those with none. The stain preferentially stains proteins containing two or more transmembrane domains (Figure 2).

In the staining protocol, after protein separation by SDS–polyacrylamide gel electrophoresis (PAGE), the gel is fixed, stained with the Pro-Q Amber reagent, washed and imaged. Stained proteins are best detected using a laser-based gel scanner. After visualizing transmembrane proteins, the total protein profile can be detected using the SYPRO Ruby protein gel stain. The Pro-Q Amber transmembrane protein gel stain is not recommended for staining proteins in 2-D, IEF or nondenaturing gels and is not suitable for staining proteins on blotting membranes.

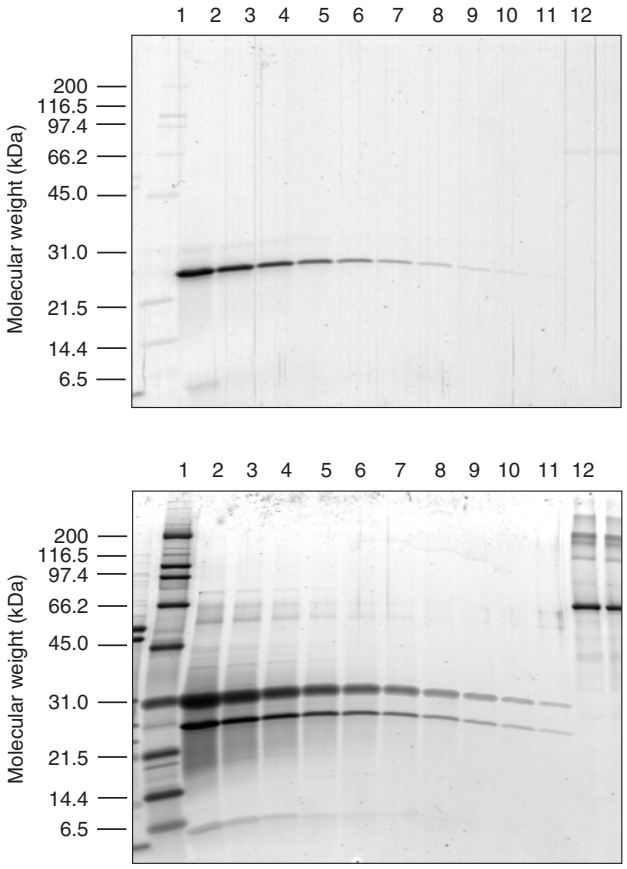

Figure 1. Sensitivity of Pro-Q Amber transmembrane protein gel stain. An SDS–polyacrylamide gel containing a twofold dilution series of the transmembrane protein standards was stained with Pro-Q Amber transmembrane protein gel stain (top) and subsequently with SYPRO Ruby protein gel stain (bottom). Lane 1, broad range molecular weight markers, 250 ng of each marker; lanes 2–11, transmembrane protein standards, twofold dilution series starting with 2 µg carbonic anhydrase and 1.4 µg bacteriorhodopsin per lane; lane 12, BSA, 250 ng. Carbonic anhydrase (upper band) is a nontransmembrane protein, and bacteriorhodopsin (lower band) is a seven-transmembrane protein. The numbers at left indicate the molecular weights of the markers. The images were acquired using a 473 nm laser–based gel scanner with a 520 nm bandpass filter for the detection of the Pro-Q Amber signal and a 580 nm bandpass filter for detection of the SYPRO Ruby signal.

Materials

Contents

- Pro-Q Amber transmembrane protein gel stain (Component A), 500 mL
- SYPRO Ruby protein gel stain (Component B), 500 mL

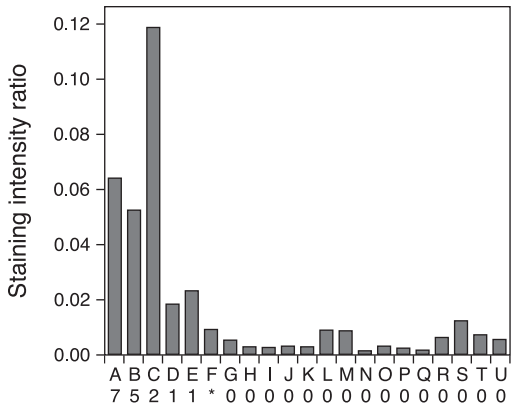

Figure 2. Ratio of Pro-Q Amber transmembrane gel stain signal to SYPRO Ruby gel stain signal for various transmembrane and nontransmembrane proteins. Proteins were separated by SDS–PAGE, stained with Pro-Q Amber transmembrane protein gel stain and imaged. The gel was then stained with SYPRO Ruby protein gel stain and imaged again. The intensity of each band was measured, and the ratio of the two signals was plotted as a bar graph. Protein A, bacteriorhodopsin; B, ATP synthase F_0 a subunit; C, ATP synthase F_0 c subunit; D, ATP synthase F_0 b subunit; E, glycophorin; F, porin; G, zein; H, carbonic anhydrase; I, ATP synthase F_1 α subunit; J, ATP synthase F_1 β subunit; K, ATP synthase F_1 γ subunit; L, ATP synthase F_1 δ subunit; M, ATP synthase F_1 ε subunit; N, myosin; O, β-galactosidase; P, phosphorylase b; Q, BSA; R, ovalbumin; S, soybean trypsin inhibitor; T, lysozyme; and U, aprotinin. The numbers along the x-axis indicate the number of α-helical transmembrane domains present in the corresponding protein; the asterisk denotes a 16-strand anti-parallel β-sheet transmembrane domain.

- Transmembrane protein standards (Component C), 25 µL of a solution containing bacteriorhodopsin (300 ng/µL) and carbonic anhydrase (500 ng/µL)
- SYPRO Ruby protein gel stain Product Information Sheet

The supplied materials are sufficient to stain ten 0.5–1 mm thick, 8 cm × 10 cm minigels.

Storage

Upon receipt, store the gel stains (Components A and B) at room temperature, protected from light. Store the transmembrane protein standards at ≤–20°C and avoid freeze-thaw cycles. When stored properly, the kit components should be stable for at least 6 months.

Materials Required but Not Provided

- Methanol
- Glacial acetic acid
- Deionized water
- Plastic staining dish (e.g., a weighing dish or a Rubbermaid® Servin' Saver container)

Protocols

Prepare the Stock Solutions

Prepare all stock solutions using deionized water (dH_2O). Stock solutions may be stored at room temperature for up to 6 months.

1.1 Prepare the fix solution. Prepare a fix solution of 50% methanol and 10% acetic acid. A 6 cm × 9 cm × 1 mm minigel requires ~200 mL of the fix solution.

1.2 Prepare the destain solution. Prepare a solution of 5% methanol and 5% acetic acid. A 6 cm × 9 cm × 1 mm minigel requires ~100 mL of the destain solution.

1.3 Prepare the transmembrane protein standards. Prepare a standards sample by adding 1 µL of the transmembrane protein standards (Component C) to 7 µL of sample buffer, and heat at 95°C for 5 minutes to denature the proteins.

Stain for Transmembrane Proteins

Perform all fixation, staining and washing steps with gentle agitation (e.g., on an orbital shaker at 50 rpm). Use a large plastic weighing dish or a reusable plastic container that has been thoroughly cleaned and rinsed with 70% ethanol before use.

2.1 Perform SDS–PAGE. Separate proteins by standard SDS–polyacrylamide gel electrophoresis.

2.2 Fix the gel. Immerse the gel in 100 mL of the fix solution (prepared in step 1.1), and incubate at room temperature with gentle agitation for at least 30 minutes.

2.3 Change the fix solution. Incubate the gel in another 100 mL of fresh fix solution at room temperature overnight. A long fixation with one change of the fix solution is necessary to completely remove the SDS from the gel. The specificity of transmembrane protein staining is reduced if the SDS is not completely removed.

2.4 Stain the gel. Incubate the gel in 50 mL of the Pro-Q Amber transmembrane protein gel stain (Component A) in the dark and with gentle agitation for 1–2 hours. Staining overnight is not recommended.

2.5 Destain the gel. Wash the gel two times in 50 mL of the destain solution (prepared in step 1.2) for 5 minutes each. Although destaining is necessary to minimize the nonspecific staining of nontransmembrane proteins, longer destaining times can decrease the fluorescent signal.

2.6 Wash the gel. Incubate the gel in ~100 mL of dH_2O for 30 minutes. Repeat this step once or twice, or until the protein standards are appropriately stained. The bacteriorhodopsin (positive control) should be strongly stained, and the carbonic anhydrase (negative control) should be only faintly visible. Quantitatively, the signal intensity of the bacteriorhodopsin band should be ≥20X that of carbonic anhydrase. If the transmembrane protein specificity is poor, then most likely the SDS was not completely removed from the gel. Place the gel in fresh fix solution for 2 hours to overnight, and then restain the gel with the Pro-Q Amber transmembrane stain.

Image and Document the Gel

The Pro-Q Amber transmembrane protein gel stain has an excitation maximum at 470 nm and an emission maximum at 570 nm. Using imaging systems with light sources and filters that match the excitation and emission maxima will provide the greatest sensitivity.

Visible-light–based scanners. To visualize the proteins, use a visible-light laser–based or xenon arc lamp–based gel-scanning instrument with an excitation maximum at 473–488 nm and a 520 nm or 580 nm longpass filter or a ~580 nm bandpass filter.

Transillumination. Stained gels can be visualized on a blue-light transilluminator, such as the Dark Reader™ transilluminator (Clare Chemical Research) or on a 300 nm UV transilluminator, but the sensitivity will be lower than with a scanning instrument. Images can be documented using either conventional or digital photography. With a Polaroid® camera and Polaroid 667 black-and-white film, use an appropriate longpass filter, such as the SYPRO photographic filter (Molecular Probes S-6656), and exposure times of 5–7 seconds. The red-orange filters typically used to photograph gels stained with ethidium bromide are not an appropriate substitute for the proper filters. For digital cameras, use a filter that corresponds closely to the emission characteristics of the stain, such as a 580 nm bandpass filter.

Stain for Total Protein

After staining with the Pro-Q Amber transmembrane protein gel stain and documenting the gel, stain the gel with the SYPRO Ruby protein gel stain (Component B, a total-protein stain). The transmembrane protein staining pattern must be viewed and documented first, because the Pro-Q Amber transmembrane stain will be washed out during the SYPRO Ruby staining protocol. Staining with SYPRO Ruby protein gel stain is described in the included literature, *SYPRO Ruby Protein Gel Stain*. A quantitative total-protein stain, such as the SYPRO Ruby protein gel stain, enables a highly abundant, nontransmembrane protein exhibiting a low level of nonspecific staining with Pro-Q Amber transmembrane stain to be distinguished from a less abundant transmembrane protein.

View and Photograph the Gel stained with SYPRO Ruby Protein Gel Stain

SYPRO Ruby protein gel stain has two excitation peaks and can be viewed using either UV illumination or blue-light illumination with a laser–based scanner.

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

Cat #	Product Name	Unit Size
M-33308	Multiplexed Proteomics™ Transmembrane Protein Gel Stain Kit *with 500 mL each of Pro-Q® Amber and SYPRO® Ruby gel stains*	1 kit

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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