Multiplexed Proteomics® Glycoprotein Gel Stain Kit with 1 L each of Pro-Q® Emerald 300 and SYPRO® Ruby gel stains

Catalog no. M33307

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Pro-Q® Emerald 300 reagent	• 30 mL	• ≤–20˚C • Desiccate • Protect from light	When stored as directed, this kit is stable for at least 6 months.
Pro-Q® Emerald 300 staining buffer and oxidizing reagent	• Pro-Q® Emerald 300 staining buffer (Component A), 1 L • Oxidizing reagent (periodic acid, MW = 227.94, Component B), 10 g	• 2–25˚C	
SYPRO® Ruby protein gel stain	• 1 L	• ≤25˚C • Protect from light	
Number of assays: Sufficient materials are supplied to stain about twenty 8 cm × 10 cm gels (0.5–1.0 mm thick) or two 20 cm × 20 cm 2-D gels (0.5–1.0 mm thick).			
Approximate fluorescence excitation/emission maxima: Pro-Q® Emerald 300 stain: 280/530 nm; SYPRO® Ruby protein gel stain: 280, 450/610 nm.			

Introduction

The Multiplexed Proteomics® Glycoprotein Gel Stain Kit provides a powerful method for differentially staining glycosylated and nonglycosylated proteins in the same gel. The technique combines our proprietary Pro-Q® Emerald 300 glycoprotein stain with our ultrasensitive SYPRO® Ruby protein gel stain. Both stains provide the simplest and most sensitive detection available, so that you can obtain more information about your glycoprotein samples in less time. In addition, they are compatible with subsequent analysis by mass spectrometry.

The Pro-Q® Emerald 300 glycoprotein stain reacts with periodate-oxidized carbohydrate groups, creating a bright green-fluorescent signal on glycoproteins. Using this stain, it is possible to detect as little as 0.5 ng of glycoprotein per band, depending upon the nature and the degree of glycosylation, making it about 50-fold more sensitive than the standard periodic acid–Schiff base method using acidic fuchsin dye. The Pro-Q® Emerald 300 glycoprotein stain also provides easier and much more reliable glycoprotein detection than mobility-shift assays, which only detect glycoproteins susceptible to specific deglycosylating enzymes (Figure 1). The green-fluorescent signal from Pro-Q® Emerald 300 stain can be visualized with 300 nm UV illumination.

The second staining method uses our proprietary SYPRO® Ruby protein gel stain to detect total protein. This easy-to-use fluorescent stain provides the same sensitivity as silver staining, but has the advantage that it does not require glutaraldehyde, which can produce false positive responses when glycoproteins are stained. The use of SYPRO® Ruby stain makes it possible to detect contaminating proteins in your sample and to easily compare the sample with molecular weight standards. For 2-D gels, total-protein staining makes it easier to localize a protein to a particular spot in the complex protein pattern. Proteins show orange-fluorescent staining when illuminated with a 300 nm UV transilluminator or a laser-based scanner with a 473 nm, 488 nm or 532 nm light source.

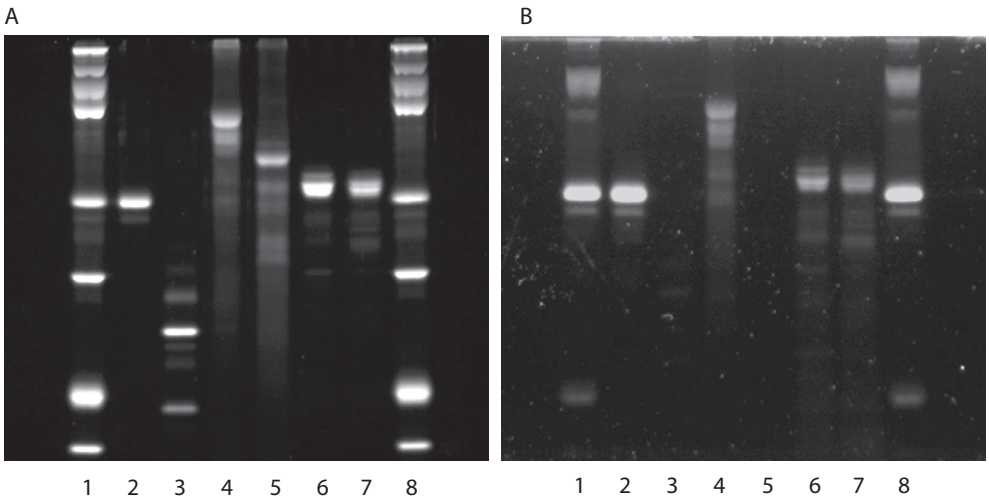

Figure 1. Mobility-shift gel assays using deglycosylating enzymes. Two identical gels were stained with SYPRO® Ruby protein gel stain (Panel A) or Pro-Q® Emerald 300 glycoprotein stain (Panel B). Each gel shows the glycoproteins α_1-acidic glycoprotein, fetuin and horseradish peroxidase (HRP) before (lanes 2, 4 and 6, respectively) and after treatment with glycosidases (lanes 3, 5 and 7, respectively). Lanes 1 and 8 contain CandyCane™ glycoprotein molecular weight standards. Treatment with the glycosidases, a mixture of endoglycosidase F, endo-O-glycosidase and sialidase, resulted in a mobility shift and loss of green-fluorescent Pro-Q® Emerald 300 staining for α_1-acidic glycoprotin and fetuin, indicating that the carbohydrate groups were cleaved off. HRP, which contains an α-(1,3)-fucosylated asparagine-N-acetylglucosamine linkage that is resistant to many glycosidases, showed no mobility shift, although the green-fluorescent Pro-Q® Emerald 300 stain revealed the presence of carbohydrate. The use of the Pro-Q® Emerald 300 stain identifies glycoproteins not susceptible to specific glycosidases, and thus provides important information about the glycoprotein's carbohydrate composition.

Before Starting

Materials Required but Not Provided

- N,N-Dimethylformamide (DMF) or dimethysulfoxide (DMSO)
- Methanol
- Glacial acetic acid
- Deionized, high quality water
- Plastic staining dish (*e.g.*, a polystyrene weighing dish)

Caution

DMSO is hazardous; avoid contact with skin and eyes and do not swallow. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials.

Experimental Protocols

General Guidelines for Gel Staining

The Multiplexed Proteomics® Glycoprotein Gel Stain Kit contains the Pro-Q® Emerald 300 reagent, a stain specific for glycoproteins, and the SYPRO® Ruby protein gel stain, a general protein stain. You should use the Pro-Q® Emerald 300 stain first and document the glycoprotein staining pattern before proceeding with SYPRO® Ruby staining.

Pro-Q® Emerald 300 Staining for Glycoproteins

The overall specificity of glycoprotein detection by the Pro-Q® Emerald 300 reagent method depends greatly upon adequate fixation and washing to remove SDS from the proteins (steps 2.2 and 2.3, below) and washing after the oxidation reaction (step 2.5) to remove residual periodate, which can interfere with staining. The protocol below provides good results for conventional gel systems. Avoid reducing the recommended incubation times and the recommended reagent volumes.

Preparing Stock Solutions

All stock solutions may be stored at room temperature for up to 6 months.

1.1 To prepare Pro-Q® Emerald 300 Stock Solution, add 24 mL of DMF to the bottle containing the Pro-Q® Emerald 300 reagent, and mix gently and thoroughly to dissolve the contents. Alternatively, you may use 24 mL of DMSO instead of DMF, although the gel background may be somewhat higher with DMSO. Store the stock solution at –20°C.

1.2 Prepare a Fix Solution of 50% methanol and 5% acetic acid in distilled water. One 8 cm × 10 cm gel requires ~200 mL of Fix Solution. One 20 cm × 20 cm 2-D gel requires 2 L of Fix Solution.

1.3 Prepare a Wash Solution of 3% glacial acetic acid in distilled water. One 8 cm × 10 cm gel requires ~1 L of Wash Solution. One 20 cm × 20 cm gel requires ~8 L of Wash Solution. An additional 1 L volume of 3% acetic acid is used in step 1.4, below.

1.4 To prepare Oxidizing Solution, add 1 L of 3% acetic acid to the bottle containing the periodic acid (Component B) and mix until the solid is completely dissolved.

Staining Procedure

The following procedure is optimized for staining 8 cm × 10 cm minigels (0.5–1.0 mm thick). Large 2-D gels (20 cm × 20 cm) require proportionally larger volumes and longer fixation and staining times, as indicated.

2.1 **Perform SDS-PAGE**. Separate proteins by standard SDS-polyacrylamide gel electrophoresis. Typically, the sample is diluted to about 10–100 µg/mL with sample buffer and 5–10 µL of diluted sample is added per lane for an 8 cm × 10 cm gel. Larger gels require proportionately more material.

2.2 **Fix the gel**. Immerse the gel in ~100 mL of Fix Solution (prepared in step 1.2) and incubate at room temperature with gentle agitation (*e.g.,* on an orbital shaker at 50 rpm) for 45 minutes. Repeat this fixation step to ensure the SDS is fully washed out of the gel. For large 2-D gels, use two 1 L volumes of Fix Solution and incubate several hours at room temperature including one overnight incubation.

2.3 **Wash the gel**. Incubate the gel in ~100 mL (~1 L for large 2-D gels) of Wash Solution (prepared in step 1.3) with gentle agitation for 10–20 minutes. Repeat this step once.

2.4 Oxidize the carbohydrates. Incubate the gel in 25–50 mL of Oxidizing Solution (prepared in step 1.4) with gentle agitation for 30 minutes. Large 2-D gels require 500 mL of Oxidizing Solution and 1 hour incubation.

2.5 Wash the gel. Incubate the gel in ~100 mL (~1 L for large 2-D gels) of Wash Solution with gentle agitation for 10–20 minutes. Repeat this step twice more (three times more for large 2-D gels).

2.6 Prepare fresh Pro-Q® Emerald 300 Staining Solution. Dilute the Pro-Q® Emerald 300 stock solution (prepared in step 1.1) 50-fold into Pro-Q® Emerald 300 staining buffer (Component B). For example, dilute 1.0 mL of the Pro-Q® Emerald 300 stock solution into 49 mL of staining buffer to make enough staining solution for one 8 cm × 10 cm gel. Large 2-D gels require 250–500 mL of staining solution.

2.7 Stain the gel. Incubate the gel **in the dark** in 25–50 mL of Pro-Q® Emerald 300 Staining Solution (made in step 2.6) while gently agitating for 90–120 minutes (250–500 mL and 2.5 hours for large 2-D gels). The signal can be seen after about 20 minutes and maximum sensitivity is reached at about 120 minutes. Do not stain overnight.

2.8 Wash the gel. Incubate the gel in ~100 mL (~1 L for large 2-D gels) of Wash Solution at room temperature for 15–20 minutes. Repeat this wash once for a total of two washes. Do not leave the gel in Wash Solution for more than 2 hours, as the staining will start to decrease. If the gel background is unacceptably high upon imaging, wash the gel a third time.

Viewing and Photographing the Gel

View and document the green-fluorescent Pro-Q® Emerald 300 staining before proceeding to stain total proteins with SYPRO® Ruby protein gel stain. The Pro-Q® Emerald 300 stain has an excitation maximum at ~280 nm and an emission maximum near 530 nm (Figure 2). You can visualize stained glycoproteins using a 300 nm UV transilluminator. The use of a photographic camera or CCD camera and the appropriate filters is essential to obtain the greatest sensitivity. The instrument's integrating capability can make bands visible that cannot be detected by eye.

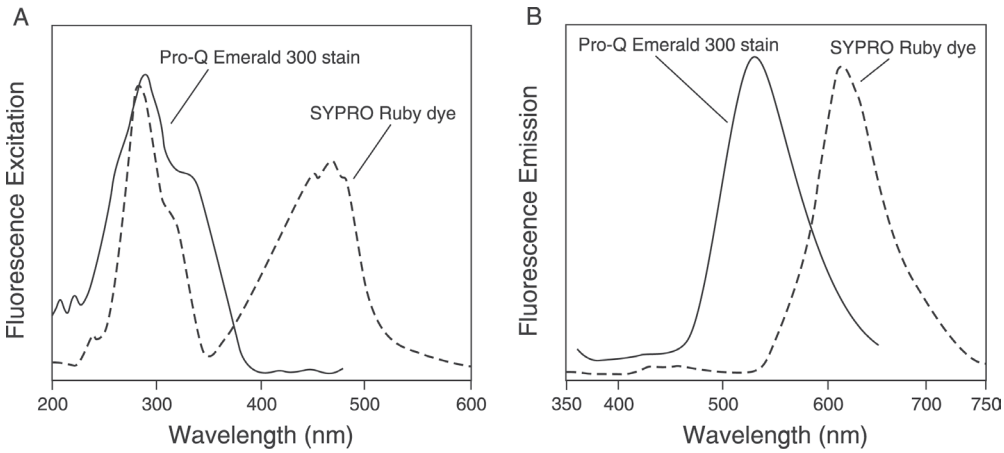

Figure 2. Excitation (A) and emission (B) spectra of Pro-Q® Emerald 300 glycoprotein reagent (solid lines) and SYPRO® Ruby protein gel stain (dashed lines).

- It is important to clean the surface of the transilluminator after each use with deionized water and a soft cloth (like cheesecloth). Otherwise, fluorescent dyes can accumulate on the glass surface and cause a high background fluorescence.
- Some fluorescent speckling may occur, especially near the edges of the gel. This speckling is an intrinsic property of the stain and does not affect sensitivity. When analyzing amounts of glycoprotein near the limit of detection, we advise running samples in the middle lanes of the gel.

- We use a 300 nm transilluminator with six 15-watt bulbs. Excitation with different light sources may not give the same sensitivity.
- When using a Polaroid® camera and Polaroid® 667 black-and-white print film, you can achieve the highest sensitivity with a 490 nm longpass filter, such as the SYPRO® protein gel stain photographic filter (Cat. no. S6656). We typically photograph minigels using an f-stop of 4.5 for 2–4 seconds, using multiple 1-second exposures.
- When using a CCD camera, you can best obtain images by digitizing at about 1024 × 1024 pixels resolution with 12-, 14- or 16-bit gray scale levels per pixel. In general, a 520 nm or 530 nm bandpass or longpass emission filter is suitable. A CCD camera–based image-analysis system can gather quantitative information that will allow comparison of fluorescence intensities between different bands or spots.
- The polyester backing on some precast gels is highly fluorescent. For maximum sensitivity when using a UV transilluminator, place the gel polyacrylamide side down and use an emission filter to screen out the blue fluorescence of the plastic.

SYPRO® Ruby Staining for Total Protein

Staining for total protein with SYPRO® Ruby protein gel stain is described in the literature included with the product SYPRO® Ruby Protein Gel Stain.

Viewing and Photographing SYPRO® Ruby Protein Gel Stain

SYPRO® Ruby protein gel stain has two excitation peaks (Figure 2A) and can be viewed using UV illumination or blue-light illumination with a laser-based scanner. For documentation, you can separate the orange-red–fluorescent SYPRO® Ruby staining from the green-fluorescent Pro-Q® Emerald 300 staining in one of two ways, described below.

- If using UV illumination, use either a longpass filter with a cutoff between 620 and 650 nm, or a bandpass filter with a center wavelength at about 645 nm, to document the SYPRO® Ruby stain alone. Filters with cutoffs at wavelengths shorter than 620 nm may show some bleedthrough of the Pro-Q® Emerald 300 signal.
- Alternatively, you can image the gel using visible-light excitation, such as used in a laser-based scanner. Visible light will excite the SYPRO® Ruby stain, but not the Pro-Q® Emerald 300 stain. You can then document the fluorescent signal from the SYPRO® Ruby stain as described in the literature included with SYPRO® Ruby Protein Gel Stain.

Subsequent Analysis by Mass Spectrometry

The SYPRO® Ruby stain involves a noncovalent interaction and will generally be removed during preparation of the sample for mass spectrometry. The Pro-Q® Emerald 300 stain only binds to carbohydrate groups at glycosylation sites. After trypsin digestion, you can directly identfy the unglycosylated peptides, which are not stained. The glycosylated peptides are difficult to identify, even under standard conditions. If necessary, they can be deglycosylated for identification by mass spectrometry.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
M33307	Multiplexed Proteomics® Glycoprotein Gel Stain Kit *with 1 L each of Pro-Q® Emerald 300 and SYPRO® Ruby gel stains*	1 kit
Related Products		
C21852	CandyCane™ glycoprotein molecular weight standards *200 gel lanes*	400 µL
C33365	Click-iT™ GalNAz metabolic glycoprotein labeling reagent (tetraacetylated *N*-azidoacetylgalactosamine) *for *O*-linked glycoproteins* *5.2 mg*	1 each
C33366	Click-iT™ ManNAz metabolic glycoprotein labeling reagent (tetraacetylated *N*-azidoacetyl-D-mannosamine) *for sialic acid glycoproteins* *5.2 mg*	1 each
C33367	Click-iT™ GlcNAz metabolic glycoprotein labeling reagent (tetraacetylated *N*-azidoacetylglucosamine) *for *O*-GlcNAc-modified proteins* *5.2 mg*	1 each
C33368	Click-iT™ *O*-GlcNAc Enzymatic Labeling System *for *N*- or *O*-linked GlcNAc glycoproteins* *10 labelings*	1 kit
M33305	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit #1 *with 1 L each of Pro-Q® Diamond (P33300) and SYPRO® Ruby (S12000) gel stains*	1 set
M33306	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit #2 *with 200 mL each of Pro-Q® Diamond (P33301) and SYPRO® Ruby (S12001) gel stains*	1 set
M33308	Multiplexed Proteomics® Transmembrane Protein Gel Stain Kit *with 500 mL each of Pro-Q® Amber and SYPRO® Ruby gel stains*	1 kit
P21855	Pro-Q® Emerald 300 Glycoprotein Gel Stain Kit *with SYPRO® Ruby protein gel stain* *10 minigels*	1 kit
P21857	Pro-Q® Emerald 300 Glycoprotein Gel and Blot Stain Kit *10 minigels or minigel blots*	1 kit
P21875	Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit *10 minigels or minigel blots*	1 kit
R33400	Rhinohide™ polyacrylamide gel strengthener concentrate *sufficient additive for 1 L of 30% acrylamide/bis-acrylamide (37.5:1)*	200 mL
R33410	Rhinohide™ Polyacrylamide Gel Strengthener Kit *makes 1 L of Rhinohide™ 30% acrylamide/bis-acrylamide (37.5:1)*	1 kit
S6656	SYPRO® photographic filter	each
S12000	SYPRO® Ruby protein gel stain	1 L
S12001	SYPRO® Ruby protein gel stain	200 mL
S21900	SYPRO® Ruby protein gel stain *bulk packaging*	5 L

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

For country-specific contact information, visit www.invitrogen.com.

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 Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology
The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.