Pro-Q® Emerald 300 Glycoprotein Gel Stain Kit with SYPRO® Ruby Protein Gel Stain (P21855)

Quick Facts

Storage upon receipt:

P33378 Pro-Q® Emerald 300 Gel Stain Kit
≤–20˚C Components

- ≤–20˚C
- Desiccate
- Protect from light

P30636 Pro-Q® Emerald 300 Gel Stain Kit
2–25˚C Components

- 2–25˚C
- DO NOT FREEZE

S12000 SYPRO® Ruby Protein Gel Stain

- ≤25˚C
- Protect from light

Ex/Em:

- 280/530 nm for Pro-Q® Emerald 300 stain
- 280, 450/610 nm for SYPRO® Ruby protein gel stain

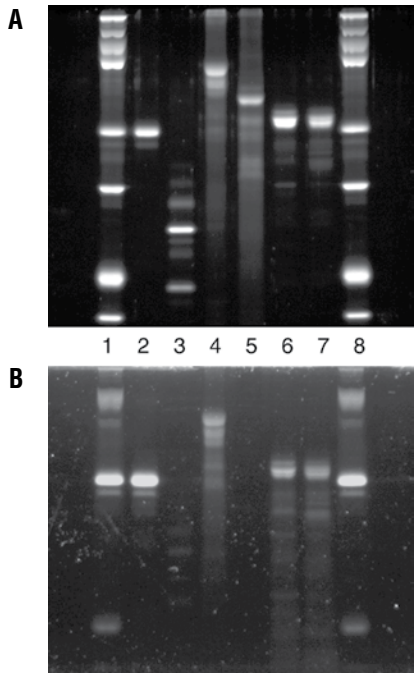

Figure 1. Mobility-shift gel assays using deglycosylating enzymes. Two identical gels were stained with either SYPRO® Ruby protein gel stain (Panel A) or Pro-Q® Emerald 300 glycoprotein stain (Panel B). Each gel shows the glycoproteins α_1-acidic glycoprotein, fetuin and horseradish peroxidase (HRP) before (lanes 2, 4 and 6, respectively) and after treatment with glycosidases (lanes 3, 5 and 7, respectively). 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 Glycoprotein Detection Kit identifies glycoproteins not susceptible to specific glycosidases, and thus provides important information about the glycoprotein's carbohydrate composition.

Introduction

Molecular Probes® Pro-Q® Emerald 300 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. 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.[1]

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 nonglycosylated 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.[2-4] 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.

The kit also includes our exclusive CandyCane™ molecular weight standards, containing a mixture of glycosylated and non-glycosylated proteins, which, when separated by electrophoresis, provide alternating positive and negative controls.

Materials

Kit Contents

The Pro-Q® Emerald 300 Glycoprotein Gel Stain Kit with SYPRO® Ruby Protein Gel stain (P21855) consists of four parts:

P33378 Pro-Q® Emerald 300 Gel Stain Kit ≤–20˚C
Components

- Pro-Q® Emerald 300 reagent (Component A), 1 vial
- CandyCane™ glycoprotein molecular weight standards (Component B), 40 µL; each protein present at ~0.5 µg/µL

P30636 Pro-Q® Emerald 300 Gel Stain Kit 2–25˚C
Components

- Pro-Q® Emerald 300 staining buffer (Component A), 250 mL
- Oxidizing reagent (Component B), 2.5 g of periodic acid

S12000 SYPRO® Ruby protein gel stain, 1L

P33380 Product Info Sheets for P21855

Sufficient materials are supplied to stain ten 8 cm × 8 cm gels (0.5–1.0 mm thick) or one 20 cm × 20 cm gel.

Storage

Upon receipt, store the P33378 components at ≤–20˚C, desiccated, and protected from light. All of the other parts to this kit (P30636, S12000, and P33380) can be stored at room temperature. Ensure that the SYPRO® Ruby protein gel stain (S12000) is protected from light. DO NOT FREEZE P30636. When stored properly, the kit should be stable for at least 6 months.

Materials Required but Not Provided

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

General Guidelines for Gel Staining

The Pro-Q® Emerald 300 Glycoprotein Gel Stain Kit provides both the Pro-Q® Emerald 300 reagent, specific for glycoproteins, and SYPRO® Ruby protein gel stain, a general protein stain. The Pro-Q® Emerald 300 stain should be used first and the glycoprotein staining pattern documented before proceeding with SYPRO® Ruby staining.

Compatibility with Invitrogen Novex® Gels

Pro-Q® Emerald 300 glycoprotein gel stain is compatible with Invitrogen Novex® Tris-glycine and Tricine gels, but it is not recommended for use with Invitrogen NuPAGE Bis-Tris.

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 should provide good results for conventional gel systems. Avoid reducing the recommended incubation times and the recommended reagent volumes.

Preparation of Stock Solutions

1.1 Pro-Q® Emerald 300 stock solution. Add 6 mL of DMF to the vial containing the Pro-Q® Emerald 300 reagent (Component A), and mix gently and thoroughly to dissolve the contents. Alternatively, 6 mL of DMSO may be used instead of DMF, although the gel background may be somewhat higher with DMSO. Store the stock solution at ≤–20°C.

1.2 Fix solution. Prepare a solution of 50% methanol and 5% acetic acid in dH_2O. One 8 cm × 8 cm gel will require ~200 mL of fix solution. One 20 cm × 20 cm gel will require 1 L of fix solution. The fix solution should be prepared fresh for each experiment.

1.3 Wash solution. Prepare a solution of 3% glacial acetic acid in dH_2O. One 8 cm × 8 cm gel will require ~1 L of wash solution. One 20 cm × 20 cm gel will require ~5 L of wash solution. An additional 250 mL volume of 3% acetic acid is used in step 1.4, below. The wash solution may be stored at room temperature for up to 6 months.

1.4 Oxidizing solution. Add 250 mL of 3% acetic acid to the bottle containing the periodic acid (Component C) and mix until completely dissolved. The oxidizing solution may be stored at room temperature for up to 6 months.

1.5 CandyCane™ molecular weight standards diluted in sample buffer. For a standard lane on an 8 cm × 8 cm gel, dilute 0.5 µL of the CandyCane™ standards (Component D) with 7.5 µL of sample buffer and vortex. This will result in ~250 ng of each protein per lane, a sufficient amount for detection of the glycoproteins by the Pro-Q® Emerald 300 stain. For a large gel, increase the amount of standard and buffer used.

Staining Procedure

The following procedure is optimized for staining 8 cm × 8 cm minigels (0.5–1.0 mm thick). A large gel (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 × 8 cm gel. A large gel requires more material.

2.2 Fix the gel. Immerse the gel in ~100 mL of fix solution (made in step 1.2) and incubate at room temperature with gentle agitation (e.g., on an orbital shaker at 50 rpm) for 30 minutes. Repeat this fixation step to ensure the SDS is fully washed out of the gel. For a large gel, use two 500 mL volumes of fix solution and fix first in 500 mL for one hour at room temperature, followed by a change of fix solution and further fixation overnight.

2.3 Wash the gel. Incubate the gel in ~100 mL (~500 mL for a large gel) of wash solution (made in step 1.3) with gentle agitation for 10–20 minutes. Repeat this step once for a small gel (twice for a large gel).

2.4 Oxidize the carbohydrates. Incubate the gel in 25 mL of oxidizing solution (made in step 1.4) with gentle agitation for 30 minutes. A large gel requires 500 mL of oxidizing solution and should be incubated for 1 hour. (The 250 mL volume of oxidizing solution from step 1.4 can be diluted with 250 mL of 3% acetic acid in order to have an adequate volume for a large gel).

2.5 Wash the gel. Incubate the gel in ~100 mL (500 mL for a large gel) of wash solution with gentle agitation for 15 minutes. Repeat this step twice more (three times more for a large gel).

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 500 µL of the Pro-Q® Emerald 300 stock solution into 25 mL of staining buffer to make enough staining solution for one 8 cm × 8 cm gel. A large gel requires 250 mL of staining solution.

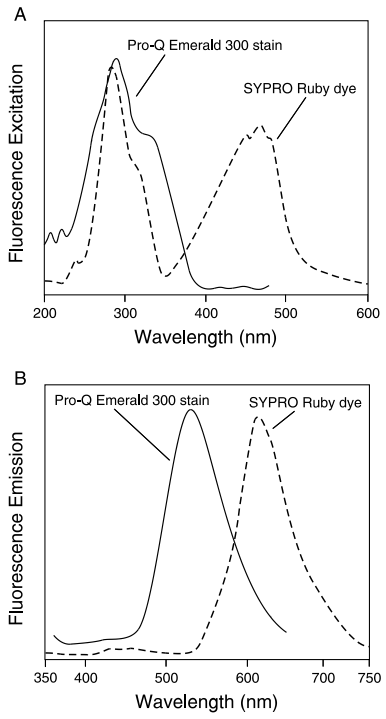

Figure 2. Excitation (A) and emission (B) spectra of Pro-Q® Emerald 300 glycoprotein reagent (solid lines) and SYPRO® Ruby protein gel stain (dashed lines).

2.7 Stain the gel. Incubate the gel in the dark in 25 mL of Pro-Q® Emerald 300 staining solution (made in step 2.6) while gently agitating for 90 minutes (250 mL and 2.5 hours for large gels). The signal can be seen after about 30 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 (500 mL for large gels) of wash solution at room temperature for 15 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, upon imaging, the gel background is unacceptably high, then wash the gel a third time.

Viewing and Photographing the Gel

The green-fluorescent Pro-Q® Emerald 300 staining should be viewed and documented before staining total proteins with SYPRO® Ruby protein gel stain. Pro-Q® Emerald 300 stain has an excitation maximum at ~280 nm and an emission maximum near 530 nm (Figure 2). Stained glycoproteins can be visualized 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.

- 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.
- Using a Polaroid® camera and Polaroid 667 black-and-white print film, the highest sensitivity is achieved with a 490 nm longpass filter, such as the SYPRO® photographic filter (S6656), available from Molecular Probes. We typically photograph minigels using an f-stop of 4.5 for 2–4 seconds, using multiple 1-second exposures.
- Using a CCD camera, images are best obtained 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 using a UV transilluminator, the gel should be placed polyacrylamide side down and an emission filter used 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 included literature, *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 either UV illumination or blue-light illumination with a laser-based scanner. For documentation, the orange-red–fluorescent SYPRO® Ruby staining can be separated 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, the gel can be imaged using visible-light excitation, such as used in a laser-based scanner. Visible light will excite SYPRO® Ruby stain, but not Pro-Q® Emerald 300 stain. The fluorescent signal from the SYPRO® Ruby stain can then be documented as described in the included literature, *SYPRO® Ruby Protein Gel Stain.*

Subsequent Analysis by Mass Spectrometry

SYPRO® Ruby stain involves a noncovalent interaction and will generally be removed during preparation of the sample for mass spectrometry. Pro-Q® Emerald 300 stain only binds to carbohydrate groups at glycosylation sites. After trypsin digestion, the unglycosylated peptides, which are not stained, can be directly identified. 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 Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
P21855	Pro-Q® Emerald 300 Glycoprotein Gel Stain Kit *with SYPRO® Ruby protein gel stain* *10 minigels*	1 kit

References

1. Proteomics 1, 841 (2001); **2.** Proteomics 5, 501 (2005); **3.** Neurosci Lett 367, 235 (2004); **4.** J Chromatogr B Analyt Technol Biomed Life Sci 793, 127 (2003).

Contact Information

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