Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit

Catalog no. P21875

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit (Cat. no. P21875) ≤–20˚C Components				
Pro-Q® Emerald 488 reagent (Component A)	10 vials	NA	• ≤–20˚C • Desiccate • Protect from light	When stored as directed, the kit components are stable for at least 6 months
CandyCane™ glycoprotein molecular weight standards (Component B)	40 µL	each protein at ~0.5 µg/µL	≤–20˚C	
Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit (Cat. no. P21875) 2–25˚C Components				
Pro-Q® Emerald 488 staining buffer (Component A)	250 mL	NA	• 2–25˚C • DO NOT FREEZE	When stored as directed, the kit components are stable for at least 6 months.
Oxidizing reagent (Component B)	2.5 g	NA		
Number of assays: Sufficient materials are supplied to stain ten 8 cm × 10 cm gels (0.5–1.0 mm thick), ten 8 cm × 10 cm blots, or one 20 cm × 20 cm 2-D gel.				
Approximate fluorescence excitation/emission maxima: Pro-Q® Emerald 488 stain: 510/520 in nm.				

Introduction

The Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit provides a powerful method for staining glycoproteins in gels or on blots. The technique employs the proprietary Pro-Q® Emerald 488 glycoprotein stain to provide a simple and highly sensitive method for glycoprotein detection. In addition, this stain is compatible with subsequent analysis by mass spectrometry.

The Pro-Q® Emerald 488 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 4 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 488 glycoprotein staining is also more sensitive and much simpler to perform than blot-based detection techniques that use biotin or digoxigenin hydrazides. The Pro-Q® Emerald 488 glycoprotein stain provides easier and much more reliable glycoprotein detection than mobility-shift assays, which only detect glycoproteins susceptible to specific deglycosylating enzymes. The green-fluorescent signal from Pro-Q® Emerald 488 stain can be visualized using visible light illumination.

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

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

- The oxidizing reagent (Component B, 2–25°C components) contains periodic acid, which is harmful if swallowed, inhaled, or absorbed through skin. It is extremely destructive to mucous membranes, the upper respiratory tract, eyes, and skin. Its inhalation may result in spasm, inflammation, and edema of the larynx and bronchi, chemical pneumonitis, and pulmonary edema.
- When handling the oxidizing reagent, always use appropriate protective equipment and practice methods to clean up spilled substances promptly. Absorb any spill onto an appropriate material, and collect and dispose of all waste in accordance with applicable laws.
- If eye or skin contact occurs, wash the affected area with water for 15 minutes and seek medical advice. If inhaled, move the individual to fresh air and seek medical advice. If swallowed, seek medical advice.

Experimental Protocols

Guidelines for Staining Glycoproteins

- You can use the Pro-Q® Emerald 488 reagent to stain glycoproteins in gels or on blots. Gel staining is faster and more sensitive, whereas blot staining provides the opportunity to combine glycoprotein staining with other blot-based detection techniques.
- 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 2.3 and 2.4) and washing after the oxidation reaction (step 2.6) to remove residual periodate, which can interfere with staining.
- Avoid reducing the recommended incubation times and the recommended reagent volumes.
- Pro-Q® Emerald 488 glycoprotein stain is compatible with general protein stains, which can be used on the same gel or blot.
- For recommendations on how to use general protein stains together with the Pro-Q® Emerald 488 reagent, refer to Staining the Gel or Blot for Total Protein, page 5.

Preparing Stock Solutions

You may store the stock solutions at room temperature for up to 6 months.

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

1.2 Wash Solution. Prepare a solution of 3% glacial acetic acid in distilled water. One 8 cm × 10 cm gel requires ~1 L of Wash Solution. One 8 cm × 10 cm blot requires ~500 mL of Wash Solution. One 20 cm × 20 cm 2-D gel requires ~8 L of Wash Solution. An additional 250 mL volume of 3% acetic acid is used in step 1.3, below.

1.3 Oxidizing Solution. Add 250 mL of 3% acetic acid to the bottle containing the periodic acid (Component B, 2–25°C components) and mix until completely dissolved.

1.4 CandyCane™ molecular weight standards diluted in sample buffer. For a standard lane on an 8 cm × 10 cm gel, dilute 0.5 µL of the CandyCane™ standards (Component B, ≤–20°C components) with 7.5 µL of sample buffer and vortex. This results in ~250 ng of each protein per lane, a sufficient amount for the detection of glycoproteins by the Pro-Q® Emerald 488 stain. For larger gels, increase the amount of the standard and the buffer used.

Staining Procedure

The following procedure is optimized for staining 8 cm × 10 cm minigels (0.5–1.0 mm thick) or 8 cm × 10 cm blots. 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 the diluted sample is added per lane for an 8 cm × 10 cm gel. Larger gels require more material.

2.2 Blot the proteins (optional). Transfer the proteins to a PVDF membrane using standard electroblotting procedures. Note that blotting is not necessary for staining glycoproteins with the Pro-Q® Emerald 488 stain; in fact, the stain has somewhat lower sensitivity on blots compared to gels.

2.3 Fix the gel or blot. Immerse the gel in ~100 mL (~25 mL for blots) of Fix Solution (prepared in step 1.1) and incubate at room temperature with gentle agitation (e.g., on an orbital shaker at 50 rpm) for at least 1 hour (at least 30 minutes for blots). Repeat this step once. For best results with gels, incubate them overnight for a second time to ensure that all of the SDS is washed out of the gel. For large 2-D gels, use two ~1 L volumes of Fix Solution, incubate at room temperature for at least 1 hour the first time, and overnight the second time.

2.4 Wash. Incubate the gel or the blot in ~100 mL (~50 mL for blots or ~1 L for large 2-D gels) of Wash Solution (prepared in step 1.2) with gentle agitation for 10–20 minutes. Repeat this step once.

2.5 Oxidize the carbohydrates. Incubate the gel or the blot in 25 mL of Oxidizing Solution (prepared in step 1.3) with gentle agitation for 20 minutes. Large 2-D gels require 500 mL of Oxidizing Solution and should be incubated for 1 hour. (You can dilute the 250 mL volume of oxidizing solution from step 1.3 with 250 mL of 3% acetic acid to have an adequate volume of Oxidizing Solution for large 2-D gels.)

2.6 Wash. Incubate the gel or the blot in ~100 mL (~50 mL for blots and ~1 L for large 2-D gels) of Wash Solution with gentle agitation for 10–20 minutes. Repeat this step two or three times more (three times more for large 2-D gels).

2.7 Prepare Pro-Q® Emerald 488 stock solution. Add 0.5 mL of DMSO or DMF (not provided) to one vial of Pro-Q® Emerald 488 reagent (Component A) and mix thoroughly. Warm the vial to room temperature before opening. Always use the Pro-Q® Emerald 488 stock solution within a few hours of preparation.

2.8 Prepare fresh Pro-Q® Emerald 488 Staining Solution. For gels, dilute the Pro-Q® Emerald 488 stock solution (prepared in step 2.7) 50-fold into Pro-Q® Emerald 488 staining buffer (Component B). For example, dilute 500 µL of the Pro-Q® Emerald 488 stock solution into 25 mL of the staining buffer to prepare enough staining solution for one 8 cm × 10 cm gel. Large 2-D gels require 250 mL of staining solution.

For blots, dilute the Pro-Q® Emerald 488 stock solution 500-fold into the Pro-Q® Emerald 488 staining buffer. For example, dilute 50 µL of the Pro-Q® Emerald 488 stock solution into 25 mL of the staining buffer to prepare enough staining solution for one 8 cm × 10 cm blot.

2.9 Stain the gel or the blot. Incubate the gel or the blot in the dark in 25 mL (250 mL for large 2-D gels) of Pro-Q® Emerald 488 Staining Solution (made in step 2.8) while gently agitating for 1.5– 2 hours (2.5 hours for large 2-D gels). The signal can be seen after about 20 minutes and maximum sensitivity is reached at about 2 hours (1 hour for blots). You may leave the gels in the staining solution overnight. Do **not** stain the blots overnight, because nonspecific staining increases over time.

2.10 Wash. Incubate the gel or the blot with ~100 mL (~50 mL for blots or ~1 L for large 2-D gels) of Wash Solution at room temperature for 15–30 minutes. Repeat this wash twice for a total of three washes, using 30–45 minutes for the second and third washes. If, upon imaging, the gel background is unacceptably high, then wash the gel a fourth time.

2.11 Rinse in water (blots only). Wash the blot twice in 25 mL of distilled water for 1 minute each.

2.12 Wash in methanol (blots only). Wash the blot in 25 mL of 100% methanol for 5 minutes. Repeat two more times. This step helps to reduce background.

2.13 Dry (blots only). If staining a blot, allow the membrane to air dry.

Viewing and Photographing the Pro-Q® Emerald 488 Glycoprotein Stain

The Pro-Q® Emerald 488 stain has an excitation maximum at ~510 nm and an emission maximum at ~520 nm. You can visualize the stained gels using visible-light illumination with wavelengths between 470 and 500 nm. **Using a photographic camera or a CCD camera and the appropriate filters is essential for obtaining the greatest sensitivity.** The instrument's integrating capability can make bands visible that cannot be detected by eye.

- Some fluorescent speckling may occur in stained gels, especially near the edges. This speckling is an intrinsic property of the stain and does not affect sensitivity. When analyzing amounts of glycoprotein near the limit of detection, run samples in the middle lanes of the gel.
- 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. Contact your camera manufacturer for recommendations on which filters to use. A CCD camera–based image-analysis system can gather quantitative information that will allow comparison of fluorescence intensities between different bands or spots.

Staining the Gel or Blot for Total Proteins

After staining with Pro-Q® Emerald 488 stain, you can stain the gel or the blot with a total protein stain, such as SYPRO® Ruby protein gel stain, SYPRO® Ruby protein blot stain, or Coomassie gold stains. Total protein staining provides valuable information about the sample, making it possible to assess the level of protein transfer to a blot, detect contaminating proteins in the sample, and 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. The fluorescent SYPRO® Ruby protein gel stain or SYPRO® Ruby protein blot stain are ideal for this purpose. These easy-to-use fluorescent stains provide high sensitivity and do not use glutaraldehyde, which can produce false positives when glycoproteins are stained.

Staining Procedure

For both gels and blots, it is important to view and document the glycoprotein staining pattern before proceeding with total protein staining. After staining the gel or the blot for total proteins, visualizing the green fluorescent Pro-Q® Emerald stain is not practical.

Subsequent Analysis by Mass Spectrometry

The Pro-Q® Emerald 488 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 website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
P21875	Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit *10 minigels or minigel blots*	1 kit

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