Pro-Q® Emerald 300 Glycoprotein Gel and Blot Stain Kit (P21857)

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

Ex/Em: 280/530 nm

Number of Assays: 10 minigels or minigel blots

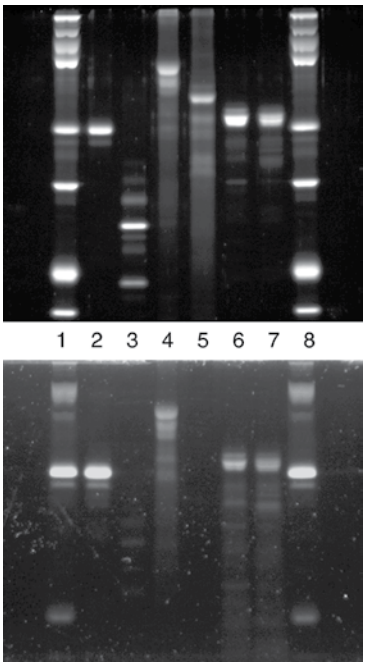

Figure 1. Mobility-shift gel assays using deglycosylating enzymes. Two identical gels were stained with either SYPRO® Ruby protein gel stain (Panel A) or Pro-Q® Emerald 300 glycoprotein stain (Panel B). Each gel shows the glycoproteins α_1-acidic glycoprotein, fetuin and horseradish peroxidase (HRP) 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 and Blot Stain Kit provides a powerful method for staining glycoproteins in gels or on blots. The technique employs our proprietary Pro-Q® Emerald 300 glycoprotein stain to provide the simplest and most sensitive method for glycoprotein detection ever developed. In addition, this stain is 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 staining is also more sensitive and much simpler to perform than blot-based detection techniques using biotin or digoxigenin hydrazides. The Pro-Q® Emerald 300 glycoprotein stain 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 is easily visualized using 300 nm UV illumination.

The kit also includes our exclusive CandyCane™ molecular weight standards, containing a mixture of glyosylated 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 and Blot Stain Kit (P21857) consists of three 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

P33379 Product Info Sheets for P21857

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.

Storage

Upon receipt, store the P33378 components at ≤–20˚C, desiccated, and protected from light. All of the other parts to this kit (P30636 and P33379) can be stored at room temperature. 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)
- Methanol
- Glacial acetic acid
- Deionized, high quality water
- Plastic staining dish (e.g., a polystyrene weighing dish)

Pro-Q® Emerald 300 Staining for Glycoproteins

Pro-Q® Emerald 300 reagent can be used to stain glycoproteins either 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, below) and washing after the oxidation reaction (step 2.6) 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.

Pro-Q® Emerald 300 glycoprotein stain is compatible with general protein stains, which can be used on the same gel or blot. Refer to *Staining the Gel or Blot for Total Protein*, below, for recommendations on how to use general protein stains together with Pro-Q® Emerald 300 reagent.

Preparation of Stock Solutions

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

1.1 Pro-Q® Emerald 300 Stock Solution. Add 6 mL of DMF to the vial containing the Pro-Q® Emerald 300 reagent 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 × 10 cm gel will require ~200 mL of Fix Solution. One 8 cm × 10 cm blot will require ~50 mL of Fix Solution. One 20 cm × 20 cm 2-D gel will require 2 L of Fix Solution.

1.3 Wash Solution. Prepare a solution of 3% glacial acetic acid in dH_2O. One 8 cm × 10 cm gel will require ~1 L of Wash Solution. One 8 cm × 10 cm blot will require ~500 mL of Wash Solution. One 20 cm × 20 cm 2-D gel will require ~8 L of Wash Solution. An additional 250 mL volume of 3% acetic acid is used in step 1.4, below.

1.4 Oxidizing Solution. Add 250 mL of 3% acetic acid to the bottle containing the periodic acid and mix until completely dissolved.

1.5 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 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 larger gels, increase the amount of standard and 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 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 Pro-Q® Emerald 300 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 (made 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 or blot. For large 2-D gels, use two 1 L volumes of Fix Solution and incubate several hours at room temperature including one overnight incubation.

2.4 Wash. Incubate the gel or blot in ~100 mL (~50 mL for blots or ~1 L for large 2-D gels) of Wash Solution (made in step 1.3) with gentle agitation for 10–20 minutes. Repeat this step once.

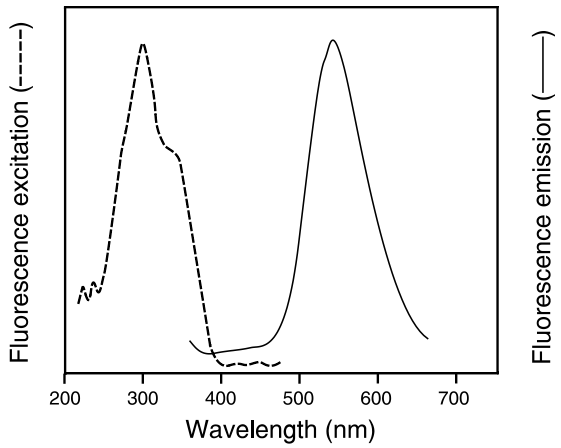

Figure 2. Excitation (dashed line) and emission (solid line) spectra of Pro-Q Emerald 300 glycoprotein reagent.

2.5 Oxidize the carbohydrates. Incubate the gel or blot in 25 mL of Oxidizing Solution (made in step 1.4) with gentle agitation for 30 minutes. Large 2-D gels require 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 large 2-D gels.)

2.6 Wash. Incubate the gel or 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 twice more (three times more for large 2-D gels).

2.7 Prepare fresh Pro-Q® Emerald 300 Staining Solution. Dilute the Pro-Q® Emerald 300 stock solution (made in step 1.1) 50-fold into Pro-Q® Emerald 300 staining buffer. 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 × 10 cm gel or blot. Large 2-D gels require 250 mL of staining solution.

2.8 Stain the gel or blot. Incubate the gel or blot in the dark in 25 mL (250 mL for large 2-D gels) of Pro-Q® Emerald 300 Staining Solution (prepared in step 2.7) while gently agitating for 90–120 minutes (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.

2.9 Wash. Incubate the gel or blot in ~100 mL (~50 mL for blots or ~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 or blot 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.

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

Viewing and Photographing the Gel or Blot

The Pro-Q® Emerald 300 stain has an excitation maximum at ~280 nm and an emission maximum at ~530 nm (Figure 2). Stained gels can be visualized using a 300 nm UV transilluminator. Stained blots are optimally visualized by illuminating the front face using a hand-held UV-B (~300 nm) light source or a top-illuminating imaging system, such as the BioRad Fluor-S™ imager. Alternatively, a UV light box can be placed on it side to illuminate the blot. *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 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, we advise running samples in the middle lanes of the gel.
- For gels, 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. Please contact your camera manufacturer for recommendations on 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.
- 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.

Staining the Gel or Blot for Total Proteins

After staining with Pro-Q® Emerald 300 stain, the gel or blot can be stained with a total protein stain, such as SYPRO® Ruby protein gel stain, SYPRO® Ruby protein blot stain, Coomassie® Blue, colloidal Coomassie Blue, silver, Ponceau S or colloidal 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. Our 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. Additionally, using these fluorescent stains, it is possible to view stained glycoproteins and total proteins simultaneously on the same gel or blot.

Staining Procedure

- For both gels and blots, it is important to view and document the glycoprotein staining pattern before proceeding with total-protein staining. Pro-Q® Emerald 300 staining is moderately quenched by SYPRO® Ruby protein stains and completely quenched by chromogenic stains.
- For best results with SYPRO® Ruby protein blot stain, wash the blot for 10–15 minutes in 100% methanol and then use the stain as outlined in the product information, *SYPRO® Ruby Protein Blot Stain*.
- For blots, an alternative double-staining procedure can be used in which SYPRO® Ruby protein blot staining is performed first, documented and then followed by Pro-Q® Emerald 300 staining. Because the SYPRO® Ruby stain washes away during Pro-Q® Emerald staining, this method results in the highest sensitivity for both stains.

Subsequent Analysis by Mass Spectrometry

The 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
P21857	Pro-Q® Emerald 300 Glycoprotein Gel and Blot Stain Kit *10 minigels or minigel blots*	1 kit

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

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.