Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit (P20495)

Quick Facts

Storage upon receipt:

P30635 Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit (P20495) ≤–20°C Components

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

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

- 2–25ºC
- DO NOT FREEZE

Ex/Em: 280/530 nm

Number of Assays: 10 minigels

Introduction

Lipopolysaccharides (LPS), also known as endotoxins, are a family of complex glycolipid molecules located on the surface of gram negative bacteria. LPS play a large role in protecting the bacterium from host defense mechanisms and antibiotics. Because of its importance in human health, intensive study is underway to understand the LPS synthesis pathways in the hopes of finding appropriate targets for designing new antimicrobial drugs.

LPS structure can be analyzed by SDS polyacrylamide gel electrophoresis, during which the heterogeneous mixture of polymers separates into a characteristic ladder pattern (Figure 1). This ladder has conventionally been detected using silver staining.[1–3] However, despite the long and complex procedures required, silver staining provides poor sensitivity and cannot differentiate LPS from proteins in the sample.

Molecular Probes Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit provides a much more powerful method for staining LPS in gels. The Pro-Q® Emerald 300 dye in the kit reacts with periodate-oxidized carbohydrate groups, creating a bright green-fluorescent signal. Using this dye, it is possible to detect as little as 200 pg of LPS in just a few hours (Figure 1). The sensitivity is at least 50–100 times that of silver staining and requires much less hands-on time. The bright green fluorescence is easy to visualize using a simple UV transilluminator.

Materials

Kit Contents

The Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit (P20495) consists of three parts:

P30635 Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit (P20495) ≤–20°C Components

- Pro-Q® Emerald 300 reagent (Component A)
- LPS standard from *Escherichia coli* serotype 055:B5 (Component B), 25 µL of a 2.5 mg/mL solution in water

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

P30637 Product Info Sheets for P20495

Sufficient materials are supplied to stain ten 8 cm × 10 cm gels, 0.5–1.0 mm thick.

Storage

Upon receipt, store the P30635 components at ≤–20°C, desiccated, and protected from light. All of the other parts to this kit (P30636 and P30637) can be stored at room temperature. DO NOT FREEZE P30636. When stored properly, the kit should be stable for at least 6 months.

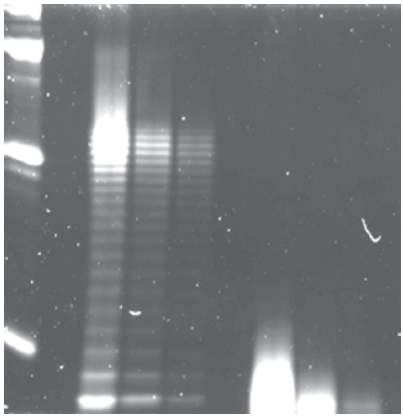

Figure 1. Analysis of lipopolysaccharides (LPS) by polyacrylamide-gel electrophoresis. LPS were electrophoresed through a 13% acrylamide gel and stained using the Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit. From left to right, the lanes contain: CandyCane™ glycoprotein molecular weight standards (~250 ng/band), blank, smooth LPS from *Escherichia coli* serotype 055:B5 (4, 1 and 0.25 µg), blank, and rough LPS from *E. coli* EH100 (Ra mutant) (4, 1 and 0.25 µg).

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

The overall success of LPS detection by the Pro-Q ® Emerald 300 reagent method depends greatly upon adequate fixation and washing to remove residual SDS from the gels (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.

Note that Pro-Q® Emerald 300 reagent will also react with glycoproteins under the conditions of this assay. To identify signals from glycoproteins, stain the gel with SYPRO® Ruby protein gel stain (S12000) after documenting the signal from the Pro-Q® Emerald 300 stain. Glycoproteins will stain with both Pro-Q® Emerald 300 stain and SYPRO® Ruby protein gel stain, whereas LPS will stain only with the Pro-Q® Emerald stain.

Preparation of Stock Solutions

Prepare all stock solutions using deionized water (dH_2O). We use water with a resistance of ≥18 megohms. 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 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.

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. 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 LPS standard. Dilute a portion of the LPS standard 10-fold into sample buffer for a final concentration of 250 µg/mL. Mix by vortexing. For a standard lane on an 8 cm × 10 cm gel, load 8 µL of the diluted LPS standard. 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). Large 2-D gels (20 cm × 20 cm) require proportionally larger volumes and longer fixation and staining times.

2.1 Perform SDS-PAGE. Separate LPS by standard SDS-polyacrylamide gel electrophoresis. Typically, the sample is diluted to about 250 µg/mL with sample buffer and 5–10 µL of diluted sample is added per lane for 8 cm × 10 cm gel. Larger gels require 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 45 minutes. Repeat this fixation step to ensure the SDS is fully washed out of the gel.

2.3 Wash the gel. Incubate the gel in ~100 mL of Wash Solution (made 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 mL of Oxidizing Solution (made in step 1.4) with gentle agitation for 30 minutes.

2.5 Wash the gel. Incubate the gel in ~100 mL of Wash Solution with gentle agitation for 10–20 minutes. Repeat this step twice more.

2.6 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 Pro-Q® Emerald 300 stock solution into 25 mL of staining buffer to make enough staining solution for one 8 cm × 10 cm gel.

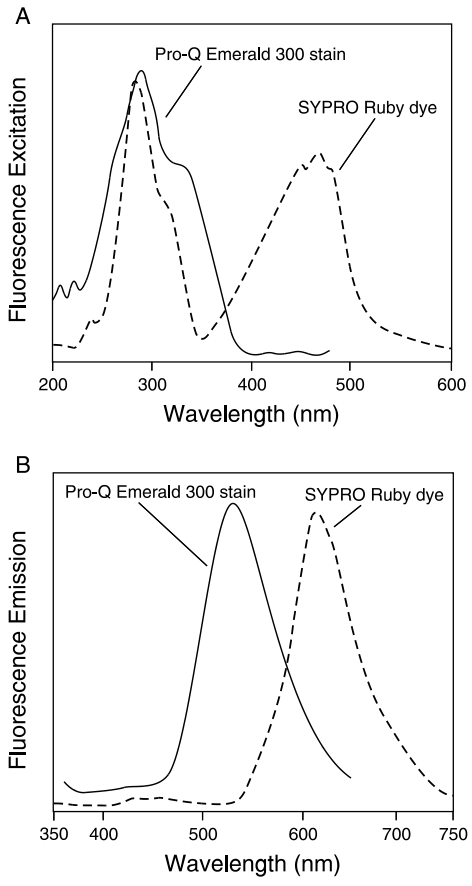

Figure 2. Excitation (A) and emission (B) spectra of Pro-Q® Emerald 300 stain (solid lines) and SYPRO® Ruby protein gel stain (dashed lines).

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 90–120 minutes. 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 with ~100 mL 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, upon imaging, the gel background is unacceptably high, then wash the gel a third time.

Viewing and Photographing the Gel

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. *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 samples containing LPS 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. 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.

Detection of Contaminating Proteins

The Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit can be used in combination with fluorescent protein stains, such as SYPRO® Ruby protein gel stain, for detection of protein contaminants. For best results, the Pro-Q® Emerald 300 stain should be used first and the LPS staining pattern documented before proceeding with fluorescent protein staining. Fluorescent protein staining may somewhat quench the Pro-Q® Emerald 300 signal.

References

1. J. Clin Microbiol 28, 2627 (1990); 2. Microbiol Immunol 35, 331 (1991); 3. J Biochem Biophys Methods 26, 81 (1993)

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

Cat #	Product Name	Unit Size
P20495	Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit *10 minigels*	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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