Revised: 08–February–2002

Pro-Q™ Sapphire 365 Oligohistidine Gel Stain (P-21876)

Quick Facts

Storage upon receipt:
- 4°C
- Protect from light

Abs/Em: 345/440 nm

Number of Assays: 20 minigels

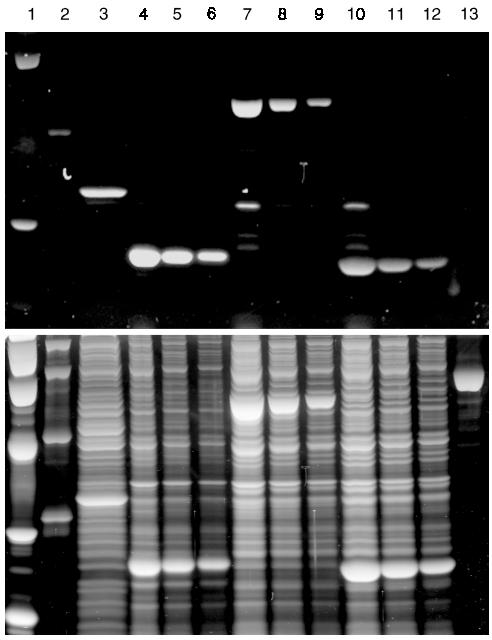

Figure 1. *Staining oligohistidine fusion proteins with Pro-Q Sapphire 365 oligohistidine gel stain. Two-fold dilutions of three different E. coli lysates, each expressing a recombinant oligohistidine fusion protein, were run on an SDS-polyacrylamide gel (lanes 4–6, 7–9 and 10–12). Lane 1 contains molecular weight standards, lane 2 contains a 6xHis protein ladder (QIAGEN), lane 3 contains a control lysate with hexahistidine–urate oxidase fusion protein (Pierce) and lane 13 contains purified BSA. After electrophoresis, the gels were stained using the Pro-Q Sapphire 365 oligohistidine gel stain (top). After documenting the oligohistidine signal, the gel was stained with SYPRO Ruby protein gel stain (bottom) for total protein.*

Introduction

The oligohistidine domain is a Ni^{2+}-binding peptide sequence comprising a string of four to six histidine residues. When the DNA sequence corresponding to the oligohistidine domain is fused in frame with a gene of interest, the resulting fusion protein can be easily purified using a nickel-chelating resin. Pro-Q™ Sapphire 365 oligohistidine gel stain provides a simple method for the detection of oligohistidine fusion proteins directly in an SDS-polyacrylamide gel (Figure 1), eliminating the need to blot the protein to a membrane. This proprietary reagent is a state-of-the-art fluorescent dye synthesized with a nitrilotriacetic acid (NTA) moiety. The NTA moiety chelates Ni^{2+} bound by oligohistidine domains. Staining is complete in just a few hours and can detect as little as ~30 ng of a hexahistidine fusion protein. We have found, however, that the sensitivity of the stain varies somewhat with the fusion protein, indicating that it may be dependent on protein context. We can typically detect 60–100 ng of oligohistidine fusion protein in a band on a minigel. Samples run on standard-sized gels with larger well sizes may exhibit lower sensitivity, as the protein will be spread out in the well. For weakly expressed proteins requiring higher sensitivity, we recommend one of our Pro-Q Oligohistidine Blot Stain Kits (P-21878 or P-21879). (Note that because the NTA is negatively charged, there may also be some weak crossreactivity with highly basic proteins.) Pro-Q Sapphire 365 stain exhibits a bright blue fluorescence when excited with UV light, (e.g., with a standard UV transilluminator). After documenting the oligohistidine signal, the gel can be stained for total protein using SYPRO® Ruby protein gel stain (Figure 1).

Materials

Contents

Pro-Q Sapphire 365 oligohistidine gel stain is provided as 500 mL of a ready-to-use solution, precomplexed with Ni^{2+}. Sufficient reagent is supplied to stain approximately twenty 8 cm × 10 cm minigels.

Storage Conditions

Upon receipt, store the reagent at 4°C, protected from light. When stored properly, the reagent should be stable for at least 6 months.

Materials Required but Not Provided

- Polystyrene staining dish
- Ethanol, spectroscopy grade
- Glacial acetic acid
- Deionized, high quality water (dH_2O)

Pro-Q Sapphire 365 Staining

The Pro-Q Sapphire 365 stain is very sensitive to the presence of dye contaminants and to SDS. For this reason, it is important to use staining dishes that are scrupulously clean or that have not been used for other stains, such as SYPRO Ruby protein gel stain, Coomassie brilliant blue or silver stain. We typically use

disposable plastic weigh boats. In addition, the solvents, volumes and times used in the fixation and wash steps must be adhered to strictly, as these steps ensure elimination of the SDS from the gel. It is extremely important to eliminate all SDS from the gel before staining. Finally, to aid in interpretation of the staining pattern, we strongly recommend using a known positive and negative control on the gel, such as those sold by Pierce Chemical Company (catalog #24572).

The following procedure is optimized for staining 0.5–0.75 mm thick, 8 cm × 10 cm minigels. For 16 cm × 18 cm gels, use twice the volume of each solution.

1.1 Prepare Fix Solution. Prepare a solution of 50% methanol and 10% acetic acid or 50% ethanol and 10% acetic acid in dH_2O. (Note that the combination of ethanol and acetic acid can result in the formation of the toxic ethyl acetate.) The use of other types of fixatives may increase nonspecific staining. One 8 cm × 10 cm gel will require ~100 mL of Fix Solution.

1.2 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.

1.3 Fix the gel. Immerse the gel in 75–100 mL of Fix Solution (made in step 1.1) and incubate at room temperature with gentle agitation (e.g., on an orbital shaker at 50 rpm) for 20 minutes. Repeat the fixation step, incubating the gel in fixative for at least 3 hours. The two sequential fixation steps are very important for removing the SDS from the gel prior to staining. Gels can be left in the fixative overnight, if desired.

1.4 Wash the gel. Wash the gel in ~50 mL of dH_2O with gentle agitation for 20 minutes. Repeat this step twice for a total of three washes.

1.5 Stain the gel. Incubate the gel in the dark in 25 mL of Pro-Q Sapphire 365 oligohistidine gel stain (or enough stain to cover the gel) while gently agitating for 45–90 minutes. Fixed gels may be left in the stain overnight.

1.6 Wash the gel. Wash the gel in ~100 mL of dH_2O at room temperature for 20 minutes. Repeat the wash step at least one more time, or until the background is no longer reduced and the signal is still strong. The optimal amount of wash time may vary from protein to protein. We recommend imaging the gel after each wash to ensure that the optimal signal is documented. Typically, between two and four 20-minute washes produces the optimal signal to backround ratio. Too little washing results in a high background, whereas excessive washing may decrease the signal. The background fluorescence may also be monitored using a hand-held UV epi-illumination source.

Viewing and Photographing the Gel

The stained oligohistidine fusion proteins can be visualized using a 300 nm or 365 nm UV transilluminator. Illumination at 365 nm provides better results; illumination at 300 nm shows more bands that stain non-specifically. *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.*

- Using a Polaroid® camera and Polaroid 667 black-and-white print film, the highest sensitivity is achieved with an appropriate filter that blocks the UV excitation light but allows the emission light (maximum at 450 nm) through to the camera. The SYPRO photographic filter (S-6656) is suitable for this purpose. Using this filter, we typically take a 10 second exposure (multiple 1-second exposures) with an f-stop of 4.5.
- 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.
- 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.
- We use a 365 nm transilluminator with six 15-watt bulbs. Excitation with different light sources may not give the same sensitivity.
- 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.

Staining the Gel for Total Protein

If desired, after documenting the Pro-Q Sapphire 365 staining, the gel can be stained using other detection methods. For instance, a total protein stain, such as SYPRO Ruby protein gel stain, may be used to visualize the total protein profile. Because the Pro-Q Sapphire 365 stain will wash away, it should be documented before beginning total protein staining.

Product List

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

Cat #	Product Name	Unit Size
P-21876	Pro-Q™ Sapphire 365 oligohistidine gel stain *20 minigels*	500 mL

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

Molecular Probes, Inc.
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 ● Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 ● Fax: (541) 344-6504 ● order@probes.com

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

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 ● Fax: (541) 465-4593 ● tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 ● Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 ● Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 ● Fax: +31-71-5241883
eurotech@probes.nl

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