Pro-Q® Sapphire 532 Oligohistidine Gel Stain (P-33354)

Quick Facts

Storage upon receipt:

- ≤6ºC
- Protect from light

Abs/Em: 535/572 nm

Number of Assays: 20 minigels

Introduction

Traditional analysis of fusion proteins containing an oligohistidine domain generally has required purification using a nickel-chelating resin followed by protein separation with SDS–polyacrylamide gel electrophoresis (PAGE) and Western blot analysis. With Molecular Probes' Pro-Q® Sapphire 532 oligohistidine gel stain, oligohistidine fusion proteins can be detected directly in an SDS–polyacrylamide gel, eliminating the need to blot the protein to a membrane (Figure 1, top). Pro-Q Sapphire 532 oligohistidine gel stain consists of a proprietary fluorescent dye selective for oligohistidine domains. Staining is complete within hours, and as little as 15 ng of a hexahistidine fusion protein can be detected. The fluorescence intensity of the stained protein varies somewhat with the fusion protein, indicating that the dye binding may be dependent on protein context. Typically, a band containing 30–50 ng of oligohistidine fusion protein can be detected in a minigel. For weakly expressed proteins requiring higher sensitivity, the Pro-Q Oligohistidine Blot Stain Kit (P-21878 or P-21879) is recommended. Samples run in a standard-size gel may be more difficult to detect since the protein is more dispersed in the larger well and thicker gel. Note that with highly basic proteins, weak crossreactivity with the reagent may occur.

Pro-Q Sapphire 532 stain with its ~535/572 nm excitation/emission maxima is optimally excited with 532 nm laser-based scanners. However, because the excitation spectrum extends broadly (Figure 2), the stain can also be detected using 473 or 488 nm blue lasers or with UV illumination. After documenting the oligohistidine signal, the gel can be stained for total protein using SYPRO® Ruby protein gel stain.

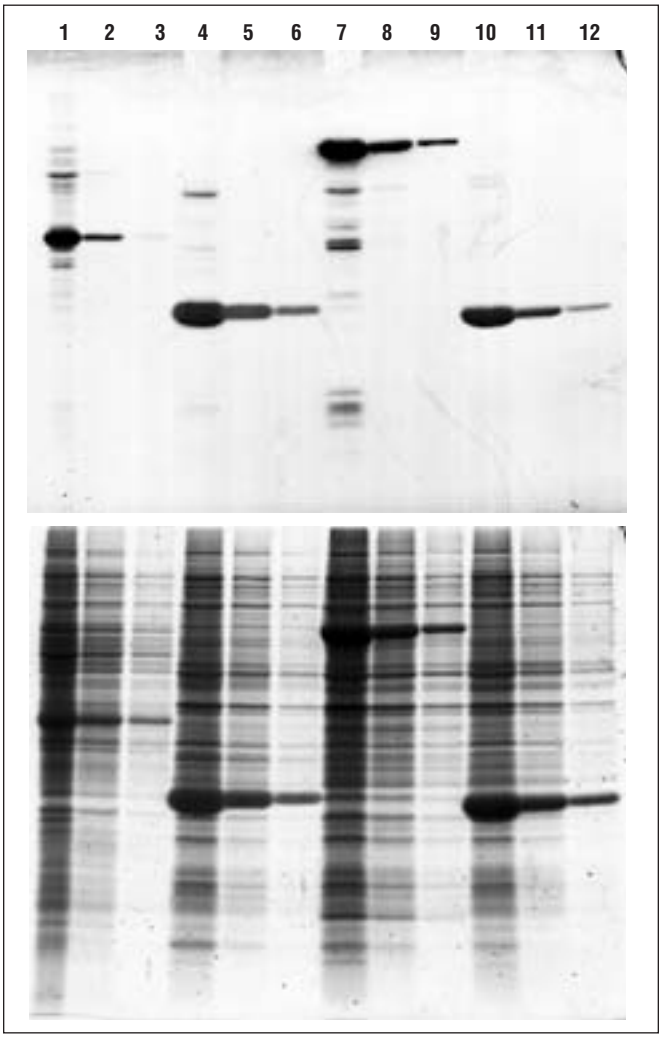

Figure 1. Staining oligohistidine fusion proteins with Pro-Q Sapphire 532 oligohistidine stain. Fourfold dilutions of four different *E. coli* lysates, each expressing a recombinant oligohistidine fusion protein, were run on an SDS–polyacrylamide gel. The lysates contained hexahistidine fusions to rat urate oxidase (lanes 1–3), to human ATP synthase OSCP subunit (lanes 4–6), to human ATP synthase α subunit (lanes 5–9) or to human ATP synthase d subunit (lanes 10–12). After electrophoresis, the gels were stained using the Pro-Q Sapphire 532 oligohistidine gel stain (top). After documenting the oligohistidine signal, the gel was stained with SYPRO Ruby protein gel stain (bottom) for total protein. For the image acquisition, excitation was with a 532 nm laser; the emission filter was a 580 nm longpass filter.

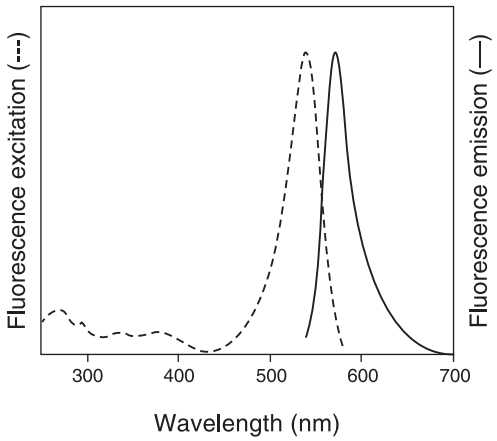

Figure 2. Fluorescence excitation and emission spectra of Pro-Q Sapphire 532 oligohistidine gel stain.

Materials

Contents

Pro-Q Sapphire 532 oligohistidine gel stain is provided in a 500 mL unit size as a ready-to-use solution. This amount of reagent is sufficient to stain approximately twenty 8 cm × 10 cm minigels.

Storage Conditions

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

Materials Required but Not Provided

- Polypropylene or polystyrene staining dish
- Ethanol or methanol
- Glacial acetic acid
- Deionized water (dH_2O)

Protocol

Since Pro-Q Sapphire 532 stain is sensitive to dye contaminants and SDS, use staining dishes, such as disposable, polystyrene weigh boats, that have not been used for other stains. All SDS must be eliminated from the gel before staining. Perform the fixation and wash steps as described, using the solvents, volumes and times indicated, to ensure elimination of the SDS from the gel. Positive and negative controls, such as the 6X His Protein Control Set from Pierce Chemical Company (catalog #24572), are strongly recommended. The following procedure is optimized for staining a 0.5 or 0.75 mm thick, 8 cm × 10 cm minigel. For a 16 cm × 18 cm gel, use twice the volume of each solution.

Fix and stain the gel

1.1 Prepare Fix Solution. One 8 cm × 10 cm gel requires ~200 mL of Fix Solution. Prepare a solution of 50% methanol and 10% acetic acid, or 50% ethanol and 10% acetic acid in dH_2O. Caution: Toxic ethyl acetate can form when ethanol and acetic acid are combined.

1.2 Separate proteins by standard SDS–PAGE. For an 8 cm × 10 cm gel, samples are typically diluted to ~10–100 µg/mL with sample buffer, and 5–10 µL of diluted sample is loaded per lane.

1.3 Fix the gel. Immerse the gel in 75–100 mL of Fix Solution (prepared in step 1.1). Incubate the gel at room temperature with gentle agitation for 20 minutes (e.g., use an orbital shaker at 50 rpm). Repeat the fixation step, and incubate the gel in fixative for at least 3 hours. The two sequential fixation steps are 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 532 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 for 20–30 minutes. Repeat the wash step two more times, and then image the gel (see below). The optimal amount of washing may vary from protein to protein. Too little washing results in a high background, whereas excessive washing may decrease the signal. If the background is too high after the three washes, wash with water for another 30 minutes and re-image the gel.

Acquire Image or Photograph the Gel

The stained oligohistidine fusion proteins are best detected using a laser-based scanning instrument with a 532 nm excitation source but can also be detected with other visible-light sources, 450 to 550 nm, or with UV light. Emission filters are ideally 555 or 580 nm longpass filters; however bandpass filters centered on 550–600 nm are also suitable.

A 300 nm transilluminator with six 15-watt bulbs can also be used. Excitation with different light sources many not give the same sensitivity. Clean the transilluminator after each use with dH_2O and a soft cloth, such as cheesecloth. This prevents the accumulation of fluorescent dyes on the glass surface that cause a high background fluorescence. To avoid the autofluorescence of the polyester backing on some precast gels, place the gel, polyacrylamide side down, on the UV transilluminator. To achieve high sensitivity using a Polaroid® camera and Polaroid 667 black-and-white print film, use a longpass or bandpass filter that blocks the UV excitation light, but allows emission light (550–600 nm) through to the camera. The SYPRO photographic filter (S-6656) works well when using an f-stop of 4.5 and a 5–6 second exposure (multiple 1-second exposures). With a CCD camera, optimal images have a resolution of about 1024 × 1024 pixels and gray scale levels of 12-, 14-, or 16-bits per pixel. In general, use a 550–600 nm bandpass filter.

Stain the Gel for Total Protein

After documenting the Pro-Q Sapphire 532 staining, the gel can be stained using other detection methods, such as SYPRO Ruby protein gel stain for visualizing the total protein profile (Figure 1, bottom). Because the Pro-Q Sapphire 532 stain can be washed out, images of Pro-Q Sapphire 532 fluorescence should be taken before staining for total protein.

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

Cat #	Product Name	Unit Size
P-33354	Pro-Q® Sapphire 532 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.

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