Pro-Q® Diamond Phosphoprotein/Phosphopeptide Microarray Stain Kit

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Pro-Q® Diamond phosphoprotein/phosphopeptide microarray stain	1 mL	• Room temperature * • Protected from light	When stored as directed, the stain should be stable for at least 6 months.
Pro-Q® Diamond microarray destain solution	500 mL		
Microarray-staining chamber with seal tabs	10 chambers		
Slide-holder tube	20 tubes		

* For long-term storage, store the kit at 2–6°C, protected from light.

Approximate fluorescence excitation/emission maxima: 555/580 nm

Introduction

The Pro-Q® Diamond phosphoprotein/phosphopeptide microarray stain kit provides a method for selectively staining phosphoproteins or phosphopeptides on microarrays. It is ideal for identification of kinase targets in signal transduction pathways and for phosphoproteomics studies. The proprietary Pro-Q® Diamond stain allows direct detection of phosphate groups attached to tyrosine, serine, or threonine residues.

Before You Begin

For best results, wear gloves and use forceps to handle slides. Prefill containers for all large-volume steps (e.g., rinsing with 10% SDS or water), then add slides to the solution. DO NOT pour solutions into slide-holder tubes containing slides, and DO NOT pour off solutions with the slides still in the tube. Use forceps to transfer slides; allow solutions to sheet off. These handling recommendations will result in consistent, even staining.

Materials Required but Not Provided

- HydroGel coated slides (product 6050018, Perkin Elmer, Inc.)
- HEPES, MOPS, or Tris buffer (PBS not recommended. See *HydroGel Coated Slides* below.)
- BSA

For the kinase reaction standard protocol only:

- ATP
- Reaction buffer
- SDS

HydroGel Coated Slides

The Pro-Q® Diamond Phosphoprotein/Phosphopeptide Microarray Stain Kit has been optimized for use with HydroGel coated slides, which are not provided as a component of this kit. HydroGel coated slides are dried prior to printing; in its dried state, the HydroGel substrate supports both noncontact and contact printing. Contact printers may require an initial adjustment of the z-position depth or substrate thickness settings (for travel of the pin head).

The HydroGel substrate is compatible with phosphate and borate buffer systems and glycerol concentrations up to 40%. Standard buffers such as MOPS and Tris may be used in place of HEPES for blocking, but phosphate-buffered systems should not be used, as residual phosphate ions may adversely affect the staining performance of the Pro-Q® Diamond stain. The HydroGel substrate is stable from pH 5 to pH 9; pH affects protein immobilization. To avoid edge effects, do not print within 1 mm of the edges of the HydroGel substrate.

Immobilization of Proteins or Peptides on HydroGel Coated Slides

Immobilization within the HydroGel substrate is a function of post-printing incubation time. Both the printing buffer used and the proteins or the peptides printed will affect the efficiency of immobilization.

After printing, incubate arrays in a humidified chamber (approximately 65%). Efficient immobilization requires a post-printing incubation of at least 8 hours in the humidity chamber; efficiency of immobilization increases as the post-printing incubation time is extended. We recommend a post-printing incubation time of at least 16 hours or more; however, the optimal post-printing incubation time should be empirically determined in order to achieve the most efficient immobilization for the specific proteins or peptides of interest. Purified thermally stable proteins should be incubated at 30°C; a lower incubation temperature may be necessary when working with unstable or impure proteins.

The proper level of relative humidity for the post-printing incubation period can be obtained by placing a saturated sodium chloride solution in the bottom of a standard incubator at 30°C. Printed slides can be stored dry at room temperature or 2–6°C for ~6 months.

For experiments involving the testing of kinase substrate specificity, follow the *Kinase Reaction Standard Protocol* before staining with Pro-Q® Diamond stain. To stain other protein or peptide arrays, skip to *Microarray Stain Protocol.*

Kinase Reaction Standard Protocol

The kinase reaction standard protocol is a suggested protocol for testing the specificity of kinases using kinase substrates spotted onto the array. These conditions may have to be optimized for your experimental conditions.

1.1 Block the slide. Place the slide in a slide-holder tube that has been prefilled with 25–30 mL of 100 mM HEPES, 1% BSA (pH 7.5). Incubate the slide in this solution at room temperature for one hour with agitation.

1.2 Rinse and dry the slide. Rinse the slide with tap water. Dry by spinning the slide briefly in a high-speed microarray centrifuge affixed with a slide holder rotor. Avoid completely drying the HydroGel pad.

1.3 Attach the gasket to the slide to form an incubation chamber. To the dry glass surface of the slide, affix one microarray staining gasket to the area surrounding the pad. Lightly place the chamber on the slide; press only the edges of the gasket to firmly attach the adhesive to the glass. Use a flat-edged object to press on the adhesive. Do not press the center of the gasket.

1.4 Add the sample. Through the portholes in the gasket, pipette purified enzyme or cell lysate with reaction buffer and ATP into the incubation chamber formed by the gasket. 100 µM ATP is a good starting concentration to use. Depending on the type of assay performed, the concentration of ATP may need to be adjusted closer to the K_m for the kinase being used. An enzyme-free or heat-inactivated negative control should be performed for comparison.

1.5 Incubate the reaction. Seal the portholes with seal tabs then place the slide in a secondary hybridization chamber such as the the CMT hybridization chamber (Corning Incorporated, Corning, NY), if available, to prevent evaporation. The secondary hybridization chamber (or the slide itself) can then be incubated at 30°C or the appropriate temperature for the kinase of interest, in an incubator or hybridization oven for 1 hour. We recommend performing small-volume incubation steps (those carried out in microarray staining chambers) on a nutator-type rotator. The optimal incubation time may need to be determined empirically.

1.6 Incubate the slide in 10% SDS. Remove the gasket using forceps and place the slide in a slide-holder tube prefilled with 10% SDS. Incubate the slide for 5 minutes with agitation. We recommend performing large-volume incubation steps on a rotisserie-type agitator. Repeat this step for a total of two SDS washes, using a freshly prefilled slide-holder tube for each wash.

1.7 Wash the slide. Transfer the slides to a container prefilled with water. Wash for 5 minutes with agitation in the water.

Repeat this water wash six more times for a total of seven washes, transferring between two containers prefilled with water before each wash.

1.8 Rinse and dry the slide. Finally, rinse the slides briefly (~20 seconds) in a steady stream of tap water and then spin them briefly in a high-speed microarray centrifuge affixed with a slide-holder rotor to remove excess water. Avoid completely drying the HydroGel pad.

Microarray Stain Protocol

This protocol describes staining of arrays printed onto the HydroGel coated slides (not provided). Pro-Q® Diamond phosphoprotein/phosphopeptide microarray stain may be used with other microarray surfaces (using different attachment chemistries), but destaining protocols may need to be optimized to reduce nonspecific background staining.

2.1 Attach the gasket to the slide to form an incubation chamber. Using sterile forceps, peel off the printed liner starting from the tab end and attach the adhesive of the microarray staining gasket to the flat, dry surface of the array. If using a microscope-sized slide, align the edges of the slide with the edges of the chamber. To ensure a secure seal, press the surface of the cover over the adhesive area with a flat-edged object. Press only the edges of the chamber adhesive; avoid pressing on the middle of the chamber.

Note: The height of the HydroGel coated pad is 4 µm when dry and can swell to 20 µm when wet. From the surface of the slide, the height of the microarray staining chamber is 0.150 mm. Although the height of the gasket should prevent the gasket from resting on the acrylamide pad, the center of the gasket sometimes presses on the center of the pad and inhibits the dispersion of pipetted solution. To avoid this, gently place the gasket on the glass surface and press only on the adhesive edge to make a tighter seal to the glass.

2.2 Add Pro-Q® Diamond microarray stain to fill the chamber. Pipet 50–60 µL Pro-Q® Diamond phosphoprotein/phosphopeptide microarray stain through one port on the seal cover while allowing air to escape through the other port. If alternate microarray surfaces are used for printing arrays and the array dimensions do not fit within the included microarray staining chamber (chamber dimensions are 22 mm × 22 mm × 0.150 mm), the volume of Pro-Q® Diamond phosphoprotein/phosphopeptide microarray stain should be scaled up or down. We do not recommend using the microarray stain for staining volumes in excess of 1 mL. If using other slides and gaskets, please test the stain carefully before performing important experiments as other adhesives and plastics may interfere with dye labeling.

2.3 Incubate the slide. Wipe away excess reagent from the surface of the chamber. Use sterile forceps to remove a seal tab from the liner strip and place a seal tab over each filling porthole. Press on both seals simultaneously, using finger pressure, to assure a secure seal. Incubate the slide in Pro-Q® Diamond microarray stain at room temperature for 30–60 minutes with agitation.

Note: All remaining steps are light sensitive. Slides and stain can be protected from light by covering with aluminum foil.

2.4 Prepare destain solution. Prior to removing the microarray staining gasket, prefill a slide-holder tube with 25–30 mL of Pro-Q® Diamond microarray destain solution and set aside.

2.5 Remove the gasket and put the slide in destain solution. To remove the microarray staining gasket, grasp the tab end along the top edge of the chamber seal and peel it away from the slide. Do not allow the slide to dry. Immediately transfer the slide to the slide-holder tube prefilled with Pro-Q® Diamond microarray destain solution.

2.6 Destain the slide. Incubate the slide for 15 minutes with agitation or rotation in 25–30 mL of Pro-Q® Diamond microarray destain solution. Repeat the destain step twice more for a total of three rounds of destaining, using a freshly prefilled slide-holder tube for each round.

2.7 Wash the slide. Prefill a slide-holder tube with 25–30 mL of distilled, deionized water. Transfer the slide to the prefilled slide-holder tube and wash for 15 minutes with agitation or rotation.

2.8 Rinse the slide. Using forceps, remove the slide and allow the water to sheet off. Retain the water in the slide-holder tube. Rinse the front and the back of the slide for 20 seconds total. Transfer the slide back to the water-filled tube.

2.9 Dry the slide. Centrifuge the slide briefly (<20 seconds) in a high-speed microarray centrifuge to dry.

Imaging Stained Slides

The excitation and emission maxima of Pro-Q® Diamond phosphoprotein/phosphopeptide microarray stain are 555 nm and 580 nm, respectively. Any microarray scanner equipped with a 532 nm or 543 nm laser can be used to excite the Pro-Q® Diamond dye. Scanners utilizing a white light arc lamp source can also be used with the appropriate bandpass excitation filter. Since the peak emission is at 580 nm, bandpass emission filters at or near 580 nm, or longpass emission filters allowing transmittance of light at and beyond 555 nm, are appropriate for capturing the fluorescence of Pro-Q® Diamond stain. Alternatively, an Alexa Fluor® 555 channel (Cy3 channel) can be used for reading the fluorescence of Pro-Q® Diamond stain.

Some microarray scanners that utilize a confocal scanning mode incorporate z-position calibration functions when scanning three-dimensional surfaces. It is appropriate and recommended to determine the optimal z-position for scanning when using the HydroGel coated slides. Determining the optimal z-position will reduce standard deviation in signal strength.

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

Cat #	Product Name	Unit Size
P33706	Pro-Q® Diamond Phosphoprotein/Phosphopeptide Microarray Stain Kit	1 kit

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