Pro-Q® Diamond Phosphoprotein Gel Stain—Tips for Success

The Instruction Manual that accompanies Pro-Q® Diamond Phosphoprotein gel stain contains detailed information on the storage and use of the Pro-Q® Diamond Phosphoprotein gel stain and on the best methods for capturing gel image data. This sheet is a distillation of the key points from that Instruction Manual, provided as a convenience for our customers.

Storage

Over time and with exposure to light, Pro-Q® Diamond Phosphoprotein gel stain will fail to perform optimally. At the time of manufacture, Molecular Probes assigns an expiration date to each lot of stain produced and applies a label to the product that specifies this date. The phosphoprotein selectivity of the stain is not guaranteed beyond this date.

It is also very important to store Pro-Q® Diamond Phosphoprotein gel stain in the dark at room temperature (or 2–6˚C for long-term storage). The 5 liter cubes are susceptible to light degradation through the spigot. It is important to store the 5 liter cubes in a cool, dark cupboard. If this is not possible, completely cover the spigot in foil when not in use.

Staining

The best results are obtained with Pro-Q® Diamond Phosphoprotein gel stain when the stain is used exactly according to the protocol. Any deviations from the protocol in the Instruction Manual can result in suboptimal staining. Scientists at Molecular Probes have also observed that while the acetonitrile/sodium acetate destain solution (formulation given in the Pro-Q® Diamond Phosphoprotein gel stain Instruction Manual) works well, the very best staining is achieved using the Pro-Q® Diamond phosphoprotein gel destaining solution.

Imaging

Many instances where Pro-Q® Diamond Phosphoprotein gel stain appears to be staining all proteins can be attributed to incorrect instrument setup. When adjusting grayscale on the documentation instrument, it is possible to make every band dark regardless of its intensity. An image can be easily optimized by focusing on a lane containing phosphorylated and nonphosphorylated standards. Details of this technique can be found in the Instruction Manual for Pro-Q® Diamond Phosphoprotein gel stain.

Quick Protocol for Pro-Q® Diamond Phosphoprotein Gel Stain

	Reagent	Standard Protocol		Rapid Protocol	
		Tris-glycine gels	NuPAGE® Bis-Tris gels[1]	Tris-glycine gels	NuPAGE® Bis-Tris gels[2]
Step 1: Fix	50% methanol, 10% acetic acid	100 mL, 30 min 2 times	100 mL, 30 min 100 mL, overnight	100 mL, 30 min 2 times	100 mL, 30 min 2 times
Step 2: Wash	Ultrapure water	100 mL, 10 min 3 times	100 mL, 10 min 3 times	100 mL, 10 min 3 times	100 mL, 15 min (bring to 60–80ºC with microwave) 4 times
Step 3: Stain	Pro-Q® Diamond stain	60 mL, 60–90 min	60 mL, 60–90 min	60 mL, microwave 40 sec to 65–80ºC, shake 7 min, microwave 20 sec to 65–80ºC, shake 7 min	60 mL, microwave ~40 sec to 65–80ºC, shake 7 min, microwave ~20 sec to 65–80ºC, shake 7 min
Step 4: Destain	Pro-Q® Diamond destain solution; or 20% acetonitrile, 50 mM sodium acetate, pH 4	80–100 mL, 30 min 3 times	80–100 mL, 30 min 3 times	80–100 mL, 30 min 2 times	80–100 mL, 30 min 2 times
Step 5: Wash	Ultrapure water	100 mL, 5 min 2 times	100 mL, 5 min 2 times	100 mL, 5 min 2 times	100 mL, 5 min 2 times
Total solution changes		11	11	10	11
Total time		4.25–4.75 hr	overnight + 3.25–3.75 hr (or 4.75–5.25 hr[1])	3.0 hr	3.5 hr (or overnight + 2.0 hr[2])

1. Alternatively, fix twice for 30 minutes followed by four 15 minute water washes microwaved to 60–80ºC.
2. Alternatively, fix 30 minutes and then overnight followed by three room temperature water washes for 10 minutes each.

Troubleshooting Pro-Q® Diamond Phosphoprotein Gel Staining

Problem	Causes	Solutions
Dark, uneven, or swirled background on gel.	Incomplete removal of destaining solution; some gel types, such as gradient gels, tend to show increased background levels toward the bottom of the gel.	Leave the gel in the final wash for 15–30 minutes, then image.
Dim fluorescent signal when the gel is photographed using UV or blue-light transillumination.	Pro-Q® Diamond–stained gels are optimally visualized using an excitation wavelength of 532–560 nm. While there is some excitation in the UV and blue-light wavelengths, it is suboptimal and results in an image 3- to 10-fold weaker than when light at 532–560 nm is used for excitation.	If UV or blue light must be used, make sure the proper photographic filter is used and the exposure time is adequate. Exposure for optimal sensitivity may need to be long enough that you can see the edge of the gel and a faint gel background in the image. For best results, use a laser-based imager with 530 nm excitation.
Streaks or speckles visible on the gel.	1) Dust. 2) Contamination of solutions used to make in-house poured gels, running buffer, or sample loading buffer. 3) Poor water quality. 4) Contamination of imager surface with fluorescent compounds. 5) Handling of gel with bare hands or contaminated or powdered gloves. 6) Staining of gel with insufficient agitation. 7) Incomplete immersion of gel during staining or destaining.	1) Avoid getting dust into solutions, on gels, and on the surface of the imaging system. 2) Use freshly made and filtered solutions. Buy precast gels if necessary. 3) Use ultrapure water (≥18 megohm-cm). Wash glassware thoroughly. 4) Clean the surface of the imaging system with 70–100% ethanol followed by ultrapure water. 5) Handle gels with clean, powder-free gloves. 6) Perform all staining, destaining, and washing incubations on an orbital shaker set at 50–60 rpm. 7) Ensure that the gel is not clinging to the side of the dish, but is completely submerged, after each solution change.
Faint or no staining of phospho-proteins (may see faint total protein staining pattern if grey scale is adjusted to higher sensitivity).	1) SDS not sufficiently removed from the gel during fixation or water wash steps. 2) Methanol and/or acetic acid not sufficiently removed during water wash step. 3) Stain has degraded because the expiration date has passed or because it has been exposed to light.	1) and 2) Include an overnight fixation as in step 3.1, or perform heated water washes as in step 4.2 (see the Pro-Q® Diamond Phosphoprotein Gel Stain Instruction Manual). 3) Check the expiration date on the label and discard the stain if the expiration date has passed. Stain should always be stored in the dark at room temperature or 4–6ºC. Five liter cubes should be stored in the dark with the spigot covered with foil when not in use.
All proteins (including all the proteins in the standard) are stained (phosphoprotein selectivity absent).	1) Stain has degraded because the expiration date has passed or because it has been exposed to light. 2) Imaging of gel was not optimized	1) Check the expiration date on the label and discard the stain if the expiration date has passed. Stain should always be stored in the dark at room temperature or 4–6ºC. Five liter cubes should be stored in the dark with the spigot covered with foil when not in use. 2) Adjust greyscale using control proteins as described in *Image Optimization* in the Pro-Q® Diamond Phosphoprotein Gel Stain Instruction Manual.

Filters recommended for use with Pro-Q® Diamond Phosphoprotein Gel Stain

Instrument (Manufacturer)	Excitation Source	Emission Filter
Typhoon Trio+, Trio, 9200, 9210, 9400, 9410 (Amersham Biosciences)	532 nm laser	560 nm longpass
FluorImager (Amersham Biosciences)	514 nm laser	570 nm bandpass
Molecular Imager FX (Bio-Rad Laboratories, Inc.)	532 nm laser	555 nm longpass
FLA-3000G, FLA-5100 (Fuji Photo Film Co, Ltd.)	532 nm laser	580 nm longpass
ProXPRESS (PerkinElmer LifeSciences, Inc.)	540/25 nm	590/30 nm