Pro-Q® Diamond Phosphoprotein Gel Stain

Table 1. Contents and storage information.

Material*	Storage†	Stability
Pro-Q® Diamond phosphoprotein gel stain	• 2–25˚C • Protect from light	When stored as directed, the stain is stable through the expiration date printed on the package.
*Pro-Q® Diamond phosphoprotein gel stain is supplied ready-to-use as a stand-alone reagent or in various convenient kit formats. See Product List for more information.		
†For long-term storage, store the stain at 2–6°C, protected from light. Store the five liter cubes in the dark. If this is not possible, keep the spigot area covered with foil when not in use.		
Approximate fluorescence excitation/emission maxima: Pro-Q® Diamond phosphoprotein stain: 555/580 in nm.		

Introduction

The Pro-Q® Diamond phosphoprotein gel stain provides a method for selectively staining phosphoproteins in polyacrylamide gels (Figure 1). It is ideal for the identification of kinase targets in signal transduction pathways and for phosphoproteomic studies.[1–3] This proprietary fluorescent stain allows direct, in-gel detection of phosphate groups attached to tyrosine, serine, or threonine residues, without the need for antibodies or radioisotopes.[1,4] The stain can be used with standard SDS-polyacrylamide gels or with 2-D gels.[5–7] The simple and reliable staining protocol delivers results in as little as 4 to 5 hours. Pro-Q® Diamond stain is fully compatible with mass spectrometry, allowing meaningful analysis of the phosphorylation state of entire proteomes.[1,3,6]

Pro-Q® Diamond phosphoprotein gel stain allows detection of as little as 1–16 ng of phosphoprotein per band, depending on the phosphorylation state of the protein. For individual phosphoproteins, the strength of the signal correlates with the number of phosphate groups and is linear over three orders of magnitude (Figure 2).[4] Pro-Q® Diamond stain has excitation/emission maxima of ~555/580 nm (Figure 3) and can be detected using a visible-light scanning instrument, a visible-light transilluminator, or (with reduced sensitivity) a 300 nm transilluminator.

Pro-Q® Diamond phosphoprotein gel stain is most useful when used in conjunction with SYPRO® Ruby protein gel stain (Cat. nos. S12000, S12001, S21900) (Figure 1).[1,4,7] SYPRO® Ruby dye is a total-protein stain that is quantitative over three orders of magnitude. Determining the ratio of Pro-Q® Diamond dye to SYPRO® Ruby dye signal intensities for each band or spot provides a measure of the phosphorylation level normalized to the total amount of protein. Using both stains in combination, it is possible to distinguish a lightly phosphorylated, high-abundance protein from a heavily phosphorylated, low-abundance protein. We also offer Pro-Q® Diamond phosphoprotein gel stain and SYPRO® Ruby protein gel stain together as convenient Multiplexed Proteomics® Phosphoprotein Gel Stain Kits.

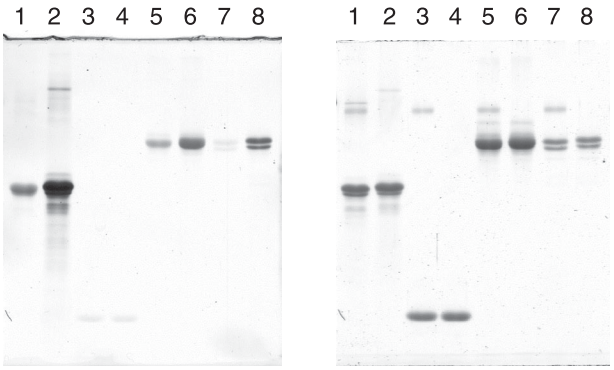

Figure 1. Selectivity of Pro-Q® Diamond phosphoprotein gel stain. A polyacrylamide gel containing various proteins was stained with Pro-Q® Diamond phosphoprotein stain (left) and subsequently with SYPRO® Ruby protein gel stain (right). The gel shows a nonphosphorylated protein, lysozyme (lanes 3 and 4), and phosphoproteins, α-casein (lanes 1 and 2), ovalbumin (lanes 5 and 6), and pepsin (lanes 7 and 8), each before (even lanes) and after (odd lanes) treatment with alkaline phosphatase. Loss of Pro-Q® Diamond staining indicates loss of all phosphates (pepsin), partial loss of phosphates (α-casein and ovalbumin), or no change (the nonphosphorylated protein, lysozyme).

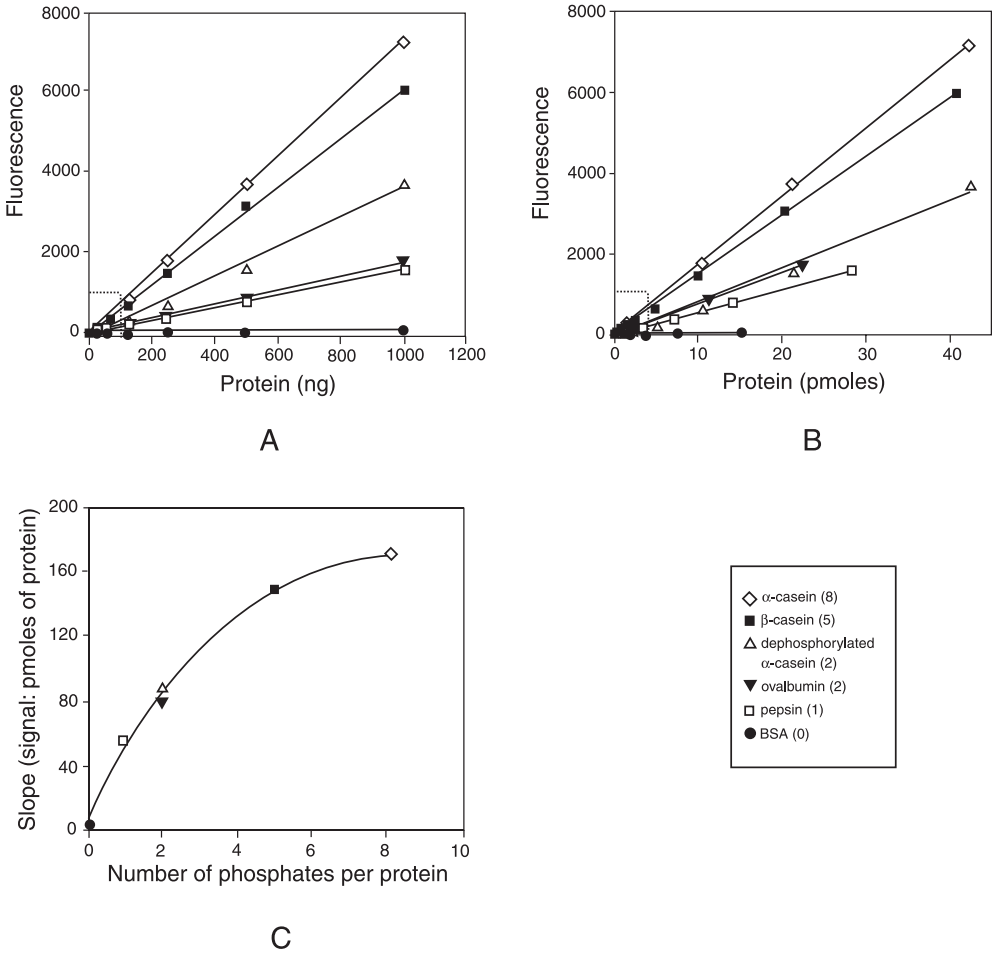

Figure 2. Sensitivity and linear range of Pro-Q® Diamond phosphoprotein gel stain. Six different proteins were serially diluted and run on separate SDS-polyacrylamide gels, then stained with Pro-Q® Diamond phosphoprotein gel stain. The images were documented on a fluorescence imager, and the fluorescence emission from each band was quantitated. The number of known phosphate groups on each protein is indicated in the figure legend. **(A)** Fluorescence emission of the band, plotted as a function of protein amount, in nanograms. **(B)** Fluorescence emission of the band, plotted as a function of picomoles of protein. **(C)** The slope of the line for each protein in panel B, plotted against the known number of phosphates per protein.

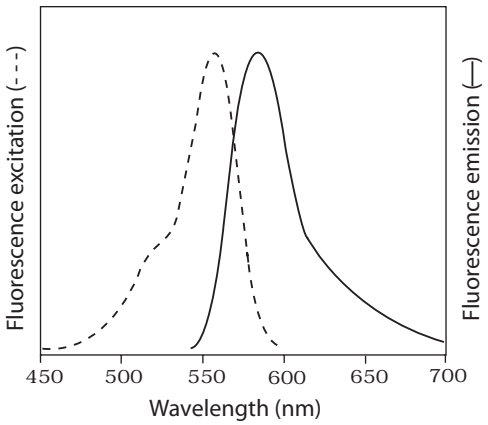

Figure 3. Excitation (dashed line) and emission (solid line) spectra of Pro-Q® Diamond phosphoprotein reagent.

Protocol Quick Guide for Experienced Users

	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 60–80°C 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 65–80°C, shake 7 min, microwave 20 sec to 65–80°C, shake 7 min	60 mL, microwave 40 sec to 65–80°C, shake 7 min, microwave ~20 sec to 65–80°C, 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 plus 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 60–80°C.
2. Alternatively, fix 30 minutes and then overnight, followed by three room temperature water washes for 10 minutes each.

Before Starting

Materials Required but Not Provided

- Reagent-grade methanol
- Glacial acetic acid
- Ultrapure water (18 megohm-cm or equivalent)
- Staining containers (polypropylene or polycarbonate)
- Destain solution (see step 1.2)
- Microwave oven (700–1200 W) (optional)
- Rotary shaker
- Powder-free latex or vinyl gloves

Preparing Stock Solutions

Prepare all stock solutions using ultrapure water (18 megohm-cm resistance recommended). You may store the stock solutions at room temperature for up to 6 months, except the fix solution, which should be prepared fresh before each use.

1.1 **Fix solution.** Prepare a solution of 50% methanol and 10% acetic acid. Prepare 200 mL fix solution per minigel (8 cm × 8 cm × 1 mm) or 1 L fix solution for each large format gel (20 cm × 20 cm × 1 mm).

1.2 **Destain solution.** There are two options for obtaining the destain solution. For optimal results, purchase Molecular Probes® proprietary Pro-Q® Diamond phosphoprotein gel destaining solution (Cat. nos. P33310, P33311) or prepare a solution of 20% acetonitrile, 50 mM sodium acetate, pH 4.0. To prepare 1 L of destain solution, combine the following and mix thoroughly:

- 50 mL of 1 M sodium acetate, pH 4.0
- 750 mL of ultrapure water
- 200 mL of acetonitrile

One 8 cm × 8 cm × 1 mm minigel requires 200–300 mL of destain solution; one large format gel (20 cm × 20 cm × 1 mm) requires ~1.5 L of destain solution.

Preparing Samples and Electrophoresis

Clean the sample

A delipidated and desalted sample is essential for adequate separation of the proteins by electrophoresis and subsequent staining by Pro-Q® Diamond phosphoprotein gel stain

2.1 For a 150 µL sample (~150–300 µg of protein), add 600 µL of methanol and mix well by vortexing.

2.2 Add 150 µL of chloroform and mix well by vortexing.

2.3 Add 450 µL of ultrapure water and mix well by vortexing.

2.4 Centrifuge at ~12,000 rpm for 5 minutes.

2.5 Discard the upper phase, keeping the white precipitation disc that forms between the upper and lower phases.

2.6 Add 450 µL of methanol and mix well by vortexing.

2.7 Centrifuge at ~12,000 rpm for 5 minutes.

2.8 Discard the supernatant and dry the pellet in a vacuum centrifuge for 10 minutes.

2.9 Resuspend the pellet in standard 1X sample buffer for electrophoresis.

Perform electrophoresis

2.10 Separate the proteins using standard polyacrylamide electrophoresis techniques. To ensure detection of less abundant phosphoproteins, use approximately the same mass of protein that you would use for a typical Coomassie blue dye–stained gel.

Staining the Gel

Guidelines for Staining

- For unambiguous interpretation of results, we recommend using the PeppermintStick™ phosphoprotein molecular weight standards (Cat. nos. P27167, P33350) in at least one gel lane.
- Perform all fixation, staining, and washing steps with gentle agitation (e.g., on an orbital shaker at 50 rpm). We recommend polycarbonate or polypropylene containers such as Rubbermaid Servin' Saver® or Stain Shield® containers; these high-density plastics adsorb only minimal amounts of the dye. It is best to use containers dedicated to Pro-Q® Diamond staining, but in any case containers should be thoroughly cleaned and rinsed with 70% ethanol before use.
- As with any fluorescent stain, during staining and subsequent wash steps, cover the gel container to exclude light.
- Adhere strictly to the volumes and times specified in this protocol for fixation, washing, staining, and destaining. Replicating the protocol is essential for consistent gel-to-gel and day-to-day comparisons.
- After obtaining results with Pro-Q® Diamond phosphoprotein gel stain, stain the gel for total protein with SYPRO® Ruby protein gel stain in order to ascertain the relative phosphorylation state of proteins. In this way, an abundant nonphosphorylated protein that exhibits low nonspecific staining with Pro-Q® Diamond stain can be distinguished from a less abundant highly phosphorylated protein. Note that nonquantitative total-protein stains, such as silver stains, are much less useful in this application. Refer to Staining the Gel for Total Protein (below) for recommendations on how to use SYPRO® Ruby protein stain in conjunction with the Pro-Q® Diamond stain.

Staining Minigels — Standard Protocol

The standard protocol works reliably for nearly all Tris-glycine SDS-PAGE minigels. If using Invitrogen NuPAGE® Bis-Tris gels, see the note below in step 3.1.

3.1 **Fix the gel.** Immerse the gel in ~100 mL of fix solution (prepared in step 1.1) and incubate at room temperature with gentle agitation for at least 30 minutes. Repeat the fixation step once more to ensure that all of the SDS is washed out of the gel. Gels can be left in the fix solution overnight.

Note: For best results with NuPAGE® Bis-Tris gels, fix overnight; alternatively, follow the instructions for fixation and washing in the rapid protocol below. To fix overnight, fix as described above for 30 minutes, then fix a second time overnight with gentle agitation..

3.2 **Wash the gel.** Incubate the gel in ~100 mL of ultrapure water with gentle agitation for 10 minutes. It is important that the gel be completely immersed in water to remove all of the methanol and acetic acid from the gel. Residual methanol or acetic acid will interfere with Pro-Q® Diamond phosphoprotein staining. Repeat this step twice, for a total of three washes.

3.3 Stain the gel. Incubate the gel in a volume of Pro-Q® Diamond phosphoprotein gel stain equivalent to 10 times the volume of the gel (e.g., 60 mL for Novex® precast minigels), with gentle agitation in the dark for 60–90 minutes. If directly comparing multiple gels, it is important that the incubation time be the same for each gel. To minimize background staining, **do not** stain overnight.

3.4 Destain the gel. Destaining is important for reducing the gel background signal and the signal from nonspecific staining. Incubate the gel in 80–100 mL of destain solution (see step 1.2) with gentle agitation for 30 minutes at room temperature, protected from light. Repeat this procedure two more times. The optimal total destaining time is about 1.5 hours.

3.5 Wash the gel. Wash twice with ultrapure water at room temperature for 5 minutes per wash. If the background is high or irregular, you may leave the gel in the second wash for 20–30 minutes and re-image.

Staining Minigels — Rapid Protocol

Heating the water washes and the stain in a microwave oven greatly shortens the required time for staining and destaining compared to the standard protocol.[8] This rapid protocol has been verified with Tris-glycine SDS-PAGE and NuPAGE® Bis-Tris gels. Volumes and incubation times were optimized for standard 8 cm × 8 cm × 1 mm minigels. Larger or thicker gels require larger volumes and longer incubation times.

4.1 Fix the gel. Immerse the gel in ~100 mL fix solution and incubate at room temperature with gentle agitation for 30 minutes. Repeat the fixation step once.

Note: For NuPAGE® Bis-Tris gels, see the note in step 4.2.

4.2 Wash the gel. Incubate the gel in ~100 mL ultrapure water at room temperature with gentle agitation for 10 minutes. Be sure the gel is completely immersed to efficiently remove the methanol and acetic acid, which will interfere with Pro-Q® Diamond staining. Repeat this step twice more, for a total of three washes.

Note: For NuPAGE® Bis-Tris gels, fix the gel twice for 30 minutes each time, then wash four times, heating the gel and water during each wash step in a microwave oven. Heat the gel in water to 70–80°C by microwaving on high power twice for ~30 seconds, agitating the water in between to distribute the heat. Remove the container from the microwave oven and agitate the gel gently in the water for 15 minutes. Repeat the heating and 15-minute agitation-incubation for a total of four heated washes. As an alternative to microwaving, you can fix the gel in 100 mL fixative solution for 30 minutes as above, then overnight in 100 mL of fix solution. Wash as in step 4.2.

4.3 Stain the gel. In a microwave-safe container, microwave a single gel in 10 times the volume of the gel (60 mL for Invitrogen Novex® precast minigels) of Pro-Q® Diamond phosphoprotein gel stain to 65–80°C about 40 seconds.

Note: Microwave ovens vary considerably; we suggest testing your oven using water and a container similar to the one you will be using.

Incubate in the dark with gentle agitation for 7 minutes. Reheat the gel and stain by microwaving to 65–80°C again (about 20 seconds). Incubate in the dark with gentle agitation for an additional 7 minutes.

4.4 Destain the gel. Destaining is important for reducing the gel background signal and the signal from nonspecific staining. Incubate the gel in 80–100 mL of destain solution (see step 1.2) with gentle agitation for 30 minutes at room temperature, protected from light. Repeat this procedure once. The total destaining time is about 1 hour.

4.5 Wash the gel. Wash twice with ultrapure water at room temperature for 5 minutes per wash. If the background is high or irregular, you may leave the gel in the second wash for an additional 20–30 minutes and re-image.

Staining Large (2-D) Gels This protocol is for 20 cm × 20 cm × 1 mm gels.

5.1 Fix the gel. Immerse the gel in ~500 mL of fix solution (prepared in step 1.1) and incubate at room temperature with gentle agitation for 30–60 minutes. Repeat the fixation overnight to ensure that all of the SDS is washed out of the gel.

5.2 Wash the gel. Incubate the gel in ~500 mL of ultrapure water with gentle agitation for 15 minutes. It is important that the gel be completely immersed in water to remove all of the methanol and acetic acid from the gel. Residual methanol or acetic acid will interfere with Pro-Q® Diamond phosphoprotein staining. Repeat this step two times for a total of three washes.

5.3 Stain the gel. Incubate the gel in the dark in 500 mL of Pro-Q® Diamond phosphoprotein gel stain with gentle agitation for 1.5–2 hours. If directly comparing multiple gels, it is important that the incubation time be the same for each gel. To minimize background staining, **do not** stain overnight.

5.4 Destain the gel. Destaining is important fo reducing the gel background signal the signal from nonspecific staining. Incubate the gel in 500 mL of destain solution (see step 1.2) with gentle agitation for 30 minutes at room temperature, protected from light. Repeat this procedure two more times. The optimal total destaining time is about 1.5 hours. You may destain the gels for longer periods, up to overnight; however, overnight destaining may result in a two- to three-fold reduction in signal intensity.

5.5 Wash the gel. Wash twice with ultrapure water at room temperature for 5 minutes per wash.

Imaging and Documenting the Gel

Pro-Q® Diamond phosphoprotein stain has an excitation maximum at ~555 nm and an emission maximum at ~580 nm (Figure 3). Imaging instruments with light sources and filters that match the excitation and emission maxima result in the highest sensitivity (see Table 2 for recommendations).

Visible-light–based scanners

Stained gels are best visualized using excitation at 532–560 nm, such as that provided with a visible-light laser–based or xenon arc lamp–based gel-scanning instrument. For most instruments, a ~580 nm longpass or ~600 nm bandpass emission filter is recommended.

Transillumination

You can visualize stained gels on a blue-light transilluminator or on a 300 nm UV transilluminator; however, the sensitivity will be 3- to 10-fold lower than with an instrument capable of 532–560 nm excitation. You can document the images using conventional or digital photography.

- With a Polaroid camera and Polaroid 667 black-and-white film, use an appropriate longpass filter, such as the SYPRO® photographic filter (Cat. no. S6656), and an exposure time of ~4 seconds. The red-orange filters typically used to photograph gels stained with ethidium bromide do not work well.
- For digital cameras, use a filter that corresponds closely to the emission characteristics of the stain, such as a 600 nm bandpass filter. For photography, optimal sensitivity may require exposures long enough that you can see the edge of the gel and a faint gel background in the image.

Table 2. 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

Staining the Gel for Total Protein

After staining with Pro-Q® Diamond stain, you can stain the gel can with a total-protein stain. SYPRO® Ruby protein gel stain is the most useful for this purpose, because it aids in determining the relative phosphorylation state of a given protein. Furthermore, for 2-D gels, SYPRO® Ruby total-protein staining makes it easier to localize a protein to a particular spot within a complex protein pattern. You must view and document the phosphoprotein staining pattern before proceeding with total-protein staining, because the Pro-Q® Diamond stain washes away during the staining procedure for total protein.

To use SYPRO® Ruby gel stain as a post-stain after Pro-Q® Diamond staining:

6.1 Image the gel for Pro-Q® Diamond stain.

6.2 Rinse the gel in ultrapure water two times for 5 minutes each (not necessary if the gel has already been rinsed in water).

6.3 Incubate the gel directly in SYPRO® Ruby gel stain solution. There is no need to repeat the fixation step.

6.4 Continue with the SYPRO® Ruby basic or rapid protocol and gel imaging, as described in the SYPRO® Ruby manual.

Image Optimization

For unequivocal identification of phosphorylated proteins, you must pay special attention to the grayscale adjustment on your digital image. When adjusting the grayscale, it is possible to make every band dark, regardless of its intensity; faint background staining of nonphosphorylated proteins can be accentuated to the point that all proteins appear stained (Figure 4).

To optimize the grayscale setting, it is essential to run known positive and negative controls in the gel. PeppermintStick™ phosphoprotein molecular weight standards (Cat. nos. P27167, P33350) contain roughly equal amounts of phosphorylated and nonphosphorylated proteins. You can easily optimize an image by focusing on the lane containing the standards and adjusting the grayscale so that the phosphorylated proteins show up as dark bands and the nonphosphorylated proteins show up only faintly (Figure 4). With a further small adjustment, bands corresponding to nonphosphorylated standards disappear; at this point, the digital image is optimized for analysis.

To account for differences in loading, or when using samples of unknown protein concentration, it is useful to further analyze the gel by staining it with SYPRO® Ruby protein gel stain and comparing the total-protein amount with the phosphoprotein amount. By performing a ratiometric analysis of each band or spot of the gel, it is possible to distinguish a large amount of minimally phosphorylated protein from a small amount of heavily phosphorylated protein. To perform the ratiometric analysis, measure the fluorescence intensity of the Pro-Q® Diamond signal (D) and the SYPRO® Ruby signal (S), and calculate the D/S ratios for 2–3 known phosphorylated control proteins as well as for proteins of interest. The ratios should be much higher for phosphoproteins (e.g., ~8.0) than for nonphosphorylated proteins (e.g., ~0.05), facilitating the distinction between specific and nonspecific signals. These numbers should be determined for each gel empirically.[3]

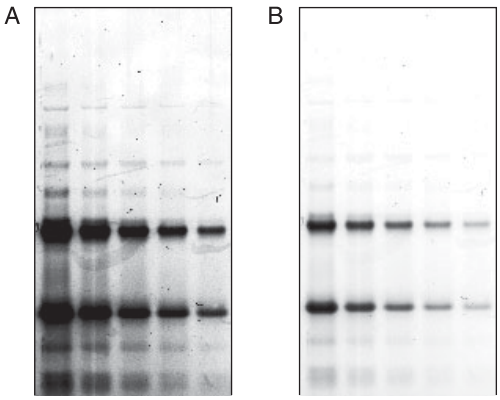

Figure 4. Optimizing the output of digital images. A dilution series of PeppermintStick™ phosphoprotein molecular weight standards was stained with Pro-Q® Diamond phosphoprotein gel stain and imaged on a laser scanner. The digital image was manipulated to illustrate improper grayscale adjustment **(A)**, then optimized to reveal the correct staining pattern **(B)**.

Disposal

Pro-Q® Diamond phosphoprotein stain is classified as an irritant. Our proprietary Pro-Q® Diamond phosphoprotein gel destaining solution is considered to be irritating to eyes and skin and a potential reproductive hazard. Acetonitrile, a component of the user-prepared destain solution, is toxic. None of these solutions should reach groundwater, a waterway, or a sewage system. We recommend that used staining and destaining solutions be mixed with flammable waste and disposed of by destructive incineration rather than by sewerage or landfill.

Troubleshooting

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 phosphoproteins (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. 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.

References

1. Electrophoresis 25, 2526 (2004); **2.** Electrophoresis 25, 2520 (2004); **3.** J Biol Chem 278, 27251 (2003); **4.** Proteomics 3, 1128 (2003); **5.** Electrophoresis 25, 2539 (2004); **6.** Electrophoresis 25, 2545 (2004); **7.** Proteomics 4, 3464 (2004); **8.** Rapid Commun Mass Spectrom 16, 272 (2002).

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

Cat. no.	Product Name	Unit Size
M33305	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit #1 *with 1 L each of Pro-Q® Diamond (P33300) and SYPRO® Ruby (S12000) gel stains*	1 set
M33306	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit #2 *with 200 mL each of Pro-Q® Diamond (P33301) and SYPRO® Ruby (S12001) gel stains*	1 set
MPM33305	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit *includes MPP33300 and S12000*	1 kit
MPM33306	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit *includes MPP33301 and S12001*	1 kit
P27167	PeppermintStick™ phosphoprotein molecular weight standards	40 µL
P33350	PeppermintStick™ phosphoprotein molecular weight standards *200 gel lanes*	400 µL
MPP33300	Pro-Q® Diamond Phosphoprotein Gel Staining Kit *includes 1 L stain and 40 µL standard*	1 kit
MPP33301	Pro-Q® Diamond Phosphoprotein Gel Staining Kit *includes 200 mL stain and 40 µL standard*	1 kit
MPP33302	Pro-Q® Diamond Phosphoprotein Gel Staining Kit *includes 5 L stain and 400 µL standard*	1 kit
P33300	Pro-Q® Diamond phosphoprotein gel stain	1 L
P33301	Pro-Q® Diamond phosphoprotein gel stain	200 mL
P33302	Pro-Q® Diamond phosphoprotein gel stain *bulk packaging*	5 L
P33310	Pro-Q® Diamond phosphoprotein gel destaining solution	1 L
P33311	Pro-Q® Diamond phosphoprotein gel destaining solution *bulk packaging*	5 L
R33400	Rhinohide™ polyacrylamide gel strengthener concentrate *sufficient additive for 1 L of 30% acrylamide/bis-acrylamide (37.5:1)*	200 mL
R33410	Rhinohide™ Polyacrylamide Gel Strengthener Kit *makes 1 L of RhinohideTM 30% acrylamide/bis-acrylamide (37.5:1)*	1 kit
S12000	SYPRO® Ruby protein gel stain	1 L
S12001	SYPRO® Ruby protein gel stain	200 mL
S21900	SYPRO® Ruby protein gel stain *bulk packaging*	5 L
S6656	SYPRO® photographic filter	each
Related Products		
A13199	annexin V, fluorescein conjugate (FITC annexin V) *100 assays*	500 µL
LC5928	BenchMark™ Fluorescent Protein Standard	125 µL
N12387	DryEase® Mini-Gel Drying System	1 kit
LC5677	Mark12™ Unstained Standard	1 mL
NP0330BOX	NuPAGE® Novex 4-12% Bis-Tris ZOOM® Gels, 10 gels	1 box
ZM0002	ZOOM® IPGRunner™ Combo Kit	1 kit
ZM0011	ZOOM® Strips, pH 3-10 NL	12 strips
ZM0018	ZOOM® Strips, pH 3-10 L	12 strips
ZM0012	ZOOM® Strips, pH 4-7	12 strips
ZM0013	ZOOM® Strips, pH 6-10	12 strips
ZM0017	ZOOM® Strips, pH 9-12	12 strips
ZS10003	ZOOM® 2D Protein Solubilizer Kit	2 x 5 mL
ZF10001	ZOOM® IEF Fractionator Combo Kit	1 kit
ZF10006	ZOOM® Basic Protein Kit	1 kit

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6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
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probesorder@invitrogen.com

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Technical Service:
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