9.3 Detection of the Total Protein Profile in Gels, on Blots and in Capillary Electrophoresis

SYPRO Protein Gel Stains

The luminescent SYPRO protein gel stains are revolutionizing the detection of the total protein profile in polyacrylamide gels. These novel protein detection reagents combine several characteristics that together make them far superior to traditional staining methods, including:

- **High sensitivity**, making it possible to detect even minimally expressed proteins
- **Fast and easy staining protocols**, simplifying the processing of multiple gels or blots
- **Minimal protein-to-protein variation in staining**, allowing quantitative comparisons between proteins\(^1\)–\(^3\)
- **Broad linear quantitation range**, an essential property for performing comparative protein expression studies
- **Compatibility with subsequent microanalysis**, streamlining techniques such as immunostaining, microsequencing and mass spectrometry\(^4\)
- **No nucleic acid staining or polysaccharide staining**, allowing analysis of relatively impure or contaminated samples
- **Instrument compatibility**, making the dyes suitable for research labs with either simple UV transilluminators or laser-based scanners

Currently the most common methods for universal profiling of proteins in gels are Coomassie brilliant blue staining\(^5\) and silver staining.\(^6\) Although Coomassie brilliant blue is an inexpensive reagent, its staining is relatively insensitive and, because it requires destaining, time consuming. Silver staining may be up to 100 times more sensitive than Coomassie brilliant blue staining, but it is relatively expensive and entails several labor-intensive steps. Silver staining also exhibits a high degree of protein-to-protein variability; staining intensity and color are very dependent on each polypeptide’s sequence and degree of glycosylation, and some proteins are detectable only as negatively stained patches. Moreover, silver staining shows very poor linearity with protein concentration (Figure 9.15) and poor reproducibility in staining from gel to gel, making it inadequate for comparative studies of protein expression in cells. The drawbacks of these traditional stains can all be overcome by using one of the SYPRO stains, without sacrificing detec-

Table 9.3 Summary of SYPRO luminescent and fluorescent protein gel stains.

<table>
<thead>
<tr>
<th>Dye Name</th>
<th>Ex/Em *</th>
<th>Major Applications</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYPRO Ruby protein gel</td>
<td>280, 450/610</td>
<td>2-D gels, IEF gels, 1-D SDS-PAGE</td>
<td>• Highest sensitivity (1–2 ng/band; comparable to silver staining)</td>
</tr>
<tr>
<td>SYPRO Orange protein gel</td>
<td>300, 470/570</td>
<td>1-D SDS-PAGE</td>
<td>• Very good sensitivity (4–8 ng/band; higher than Coomassie brilliant blue staining)</td>
</tr>
<tr>
<td>SYPRO Red protein gel</td>
<td>300, 550/630</td>
<td>1-D SDS-PAGE</td>
<td>• Very good sensitivity (4–8 ng/band; higher than Coomassie brilliant blue staining)</td>
</tr>
<tr>
<td>SYPRO Tangerine protein gel</td>
<td>300, 490/640</td>
<td>1-D SDS-PAGE, blotting applications, zymography, electrophoresis</td>
<td>• Very good sensitivity (4–8 ng/band; higher than Coomassie brilliant blue staining)</td>
</tr>
</tbody>
</table>

* Excitation (Ex) and emission (Em) maxima, in nm. For maximum sensitivity, use excitation sources and optical filters matched to these values.

Our SYPRO Ruby protein gel and blot stains are premiere reagents for quantitative proteomics that are fully compatible with Edman sequencing and mass spectrometry. Unlike labor-intensive silver staining, which requires multiple steps that must be carefully timed, SYPRO Ruby protein gel staining requires only sample fixation, staining and a final wash step to provide sensitivity similar to that of silver staining but with far better detection linearity and a greater dynamic range.

Figure 9.12 Amounts of carbonic anhydrase ranging from 1 ng to 1000 ng were separated on an SDS-polyacrylamide gel and stained with the SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900). The inset shows the excellent linearity in the lower part of the range from 1 ng to 60 ng protein. Staining intensities were quantitated using the Bio-Rad Molecular Imager FX System. For comparison, the gray band shows the linear range for the same protein detected with silver staining.
tion sensitivity. We have developed a SYPRO dye optimized for protein profiling in nearly every type of gel (Table 9.3) or blot application (Table 9.4). The characteristics and applications of the individual SYPRO protein gel and blot stains for detecting the total protein profile of a sample are described in this section. Section 9.4 discusses combinations of these SYPRO stains with additional reagents for selective detection of proteins in the sample, in what we term Multiplexed Proteomics (MP).

**SYPRO Ruby Protein Gel Stain: Ultrasensitive Protein Detection in 1-D, 2-D and IEF Gels**

Our patented SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900) is a ready-to-use protein stain that has sensitivity equal to or exceeding that of the best silver staining techniques, is compatible with Edman sequencing and mass spectrometry and can be visualized with a simple UV transilluminator or a laser-based scanner. The SYPRO Ruby protein gel stain has characteristics that make it far superior to conventional staining techniques:

- **High-sensitivity staining.** SYPRO Ruby protein gel stain provides at least the same subnanogram sensitivity as the best silver staining techniques in 1-D, 2-D or IEF gels (Figure 9.12).
- **Simple protocol.** After fixation, the gel is incubated in the staining solution (Figure 9.13). No stop solutions or destaining steps are required and, unlike silver staining, gels can be left in the dye solution for indefinite periods without overstaining, vastly simplifying the simultaneous processing of multiple gels and making it possible to perform high-throughput staining without investing in robotic staining devices.
- **Accurate peptide and protein detection.** SYPRO Ruby protein gel stain shows little protein-to-protein variability in staining and detects some proteins that are completely missed by silver staining (Figure 9.14), such as heavily glycosylated proteins. Unlike silver staining, SYPRO Ruby dye does not stain extraneous nucleic acids, lipids or carbohydrates in the sample.
- **Excellent performance in comparative protein expression studies.** SYPRO Ruby stain shows a greater linear quantitation range than either silver or Coomassie brilliant blue staining — extending over three orders of magnitude — making it possible to accurately compare protein expression levels (Figure 9.12, Figure 9.15). Gel-to-gel staining is extremely consistent; same-spot intensity comparisons between identical 2-D gels show a correlation coefficient of 0.9. Multiple gels can easily be compared using available software (Figure 9.16).

**Figure 9.13** Staining gels with SYPRO Ruby protein gel stain is simple: just fix, stain and wash.

**Figure 9.14** SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900) shows less protein-to-protein variation than silver staining. Proteins from a cell lysate were run on a 2-D gel and stained with SYPRO Ruby protein gel stain (left) or silver stain (right). The grayscale values of the SYPRO Ruby dye-stained gel have been inverted for easier comparison with the silver-stained gel.

**Figure 9.16** Comparison of two protein samples run on 2-D gels. Proteins from either a normal liver tissue sample or a liver tumor sample were run on two 2-D gels and stained with SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900). Images of the gels were captured using the FLA-3000 scanner (Fujifilm). Images from a portion of the two gels were then pseudocolored either pink or green, overlaid and matched spot-for-spot using Z3 software (Compugen). Green spots represent proteins expressed in the liver tumor samples; pink spots represent proteins expressed in the normal liver tissue sample. Black spots represent proteins expressed in both tissues.
No other protein quantitation method, including running multiple prestained samples on the same gel, gives results that approach this level of discrimination.18

- **Compatibility with microsequencing and mass spectrometry.** Unlike silver staining techniques, which use glutaraldehyde- or formaldehyde-based fixatives, SYPRO Ruby dye is a gentle stain that interacts noncovalently with proteins. Thus, high-quality Edman sequencing or mass spectrometry data4,11,12,19 (Figure 9.17) can be obtained immediately after staining, without modification steps that may compromise sensitivity.

- **Utility for isoelectric focusing (IEF).** SYPRO Ruby protein gel stain also provides reliable, high-sensitivity staining for isoelectric focusing (IEF) gels (Figure 9.18) without the problems typically encountered with silver staining, such as ampholyte staining or mirroring effects on the plastic gel backing.

- **Easily visualized signal.** SYPRO Ruby protein gel stain comprises the transition metal ruthenium, which shows an extremely bright and photostable red-orange luminescence when excited with either UV or blue light (Figure 9.19). Stained proteins can be visualized using a UV transilluminator, a blue-light transilluminator or a laser-based scanning instrument. Gels can then be documented using Polaroid 667 black-and-white print film, a CCD camera with an image documentation system or a laser-based scanning instrument. For optimal sensitivity using a UV transilluminator and Polaroid 667 black-and-white print film, the SYPRO photographic filter (S-6656, Figure 24.51) is recommended.

- **Minimal hazardous waste.** The amount of hazardous waste generated is greatly reduced compared to silver staining, minimizing the hassles and expense associated with waste disposal.

Figure 9.15 Quantitation of proteins in gels using SYPRO Ruby protein gel stain versus silver stain. Dilutions of proteins were electrophoresed on eight different SDS-polyacrylamide gels, two gels for each of four dilution ranges. The gels were stained with either SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900) or a silver stain. Staining intensities were quantitated using either the Fluor-S image documentation system (Bio-Rad) or the FLA-3000 (Fuji) and plotted against the protein amount for bovine serum albumin. SYPRO Ruby protein gel stain shows a linear quantitation range over three orders of magnitude, and consistent staining intensities from gel to gel. In contrast, the silver stain shows linear quantitation over only a small range, a very shallow slope and inconsistent staining intensities from gel to gel, even when corrected for background differences.
SYPRO Ruby protein gel stain\textsuperscript{7,10–12,14,16,20,21} is supplied as 200 mL of a 1× staining solution (S-12001), sufficient for staining about four minigels, or 1 L of a 1× staining solution (S-12000), sufficient for staining about 20 minigels or two standard 2-D gels. Additionally, we offer SYPRO Ruby protein gel stain in a 5 L box (S-21900), sufficient for staining about 100 minigels or 10 standard 2-D gels. These boxes are easy to stack and store, and the convenient spigot makes it easy to dispense just the right amount of stain (Figure 9.20). Significant discounts are available for multiple-unit purchases of the SYPRO Ruby products. All of the SYPRO Ruby protein gel stains are accompanied by detailed instructions for staining and photography of gels.

**SYPRO Orange and SYPRO Red Protein Gel Stains: For Routine Detection of Proteins in 1-D SDS-Polyacrylamide Gels**

Molecular Probes’ patented SYPRO Orange (S-6650, S-6651) and SYPRO Red (S-6653, S-6654) protein gel stains provide a fluorescence-based alternative for protein detection in SDS-polyacrylamide gels that is not only faster and more sensitive than Coomassie brilliant blue staining, but can be as sensitive as short-protocol silver staining methods (Figure 9.21) at a fraction of the time, effort and cost of silver staining.\textsuperscript{1,22–25} In the presence of excess SDS, nonpolar regions of polypeptides are coated with detergent molecules, forming a micelle-like structure with a nearly constant SDS/protein ratio (1.4 g SDS:1.0 g protein); this constant charge-per-mass ratio is the basis of molecular weight determination by SDS-polyacrylamide gel electrophoresis.\textsuperscript{26} These SYPRO dyes appear to bind to the SDS coat that surrounds proteins in SDS-polyacrylamide gels. Thus, the staining observed with the SYPRO Orange or SYPRO Red dyes exhibits relatively little protein-to-protein variation and is linearly related to protein mass (Figure 9.22). Some important features of the SYPRO Orange and SYPRO Red protein gel stains include:

- **Ease of use.** Following electrophoresis, the gel is stained for 10–60 minutes and then briefly rinsed — no separate fixation, stop or destaining steps are required. After staining, the gel is immediately ready for photography, or it can be stored, in or out of the staining solution, for days.

- **High sensitivity.** The SYPRO Orange or SYPRO Red protein gel stains routinely provide a sensitivity level of at least 8–16 ng per band in SDS-polyacrylamide gels when visualized with standard 300 nm transillumination (Figure 9.21). Photography, using Polaroid 667 black-and-white print film and a SYPRO photographic filter (S-6656, Figure 24.51), enhances the detection of staining with the SYPRO Orange or SYPRO Red dyes by several-fold over visible detection because the film integrates the signal throughout the duration of the exposure. Laser-based scanners also detect nanogram quantities of SYPRO dye–stained proteins in gels.

- **Broad linear quantitation range.** Protein detection in gels stained with either the SYPRO Orange or SYPRO Red stain is linear over three orders of magnitude in protein quantity\textsuperscript{25} (Figure 9.22).

- **Uniform protein staining.** Unlike silver staining,\textsuperscript{27} the SYPRO Orange and SYPRO Red dyes exhibit relatively low protein-to-protein variability in SDS-polyacrylamide gels\textsuperscript{1} (Figure 9.22) and do not stain nucleic acids, which are sometimes found in protein mixtures from cell or tissue extracts.\textsuperscript{28} In addition, the SYPRO Orange and SYPRO Red dyes only weakly stain lipopolysaccharides in bacterial lysates, whereas these biopolymers are strongly detected by some types of silver staining.\textsuperscript{25} Glycoproteins (such as the IgG variable subunit) and proteins with prosthetic groups (such as bovine cytochrome oxidase) are also efficiently stained with the SYPRO Orange or SYPRO Red dyes.\textsuperscript{1}

- **Photostability.** Proteins stained with the SYPRO Orange or SYPRO Red dyes are relatively photostable, enabling the researcher to acquire multiple photographic images and to use long film-exposure times (2–8 seconds). Gels that are illuminated for long periods of time may partially photobleach but can be restained with little loss of sensitivity.\textsuperscript{28}

- **Compatibility with many types of instruments.** Although their excitation maxima are in the visible range (Figure 9.23), SYPRO dye–stained gels are readily visualized using standard 300 nm transilluminators.\textsuperscript{22} SYPRO Orange protein gel stain also exhibits good sensitivity when viewed with a blue-light transilluminator, and both

![Figure 9.17 Mass spectrum profile of NADH:ubiquinone reductase precursor (75,000-dalton subunit) obtained after 2-D gel electrophoresis of bovine heart mitochondria and staining with SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900). Bovine heart mitochondria were a gift of Roderick Capaldi, University of Oregon.](image1)

![Figure 9.18 SYPRO Ruby protein gel stain versus silver stain for IEF gels. Serial dilutions of isoelectric focusing protein standards were electrophoresed on two identical polyacrylamide gels. One gel was stained with SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900) (left) and the other with silver stain (right). Both stains show a similar limit of sensitivity for all proteins.](image2)

![Figure 9.19 Excitation (dashed line) and emission (solid line) spectra of the SYPRO Ruby protein gel and blot stains (S-11791, S-12000, S-12001, S-21900).](image3)

![Figure 9.20 Excitation (dashed line) and emission (solid line) spectra of the SYPRO Ruby protein gel and blot stains (S-11791, S-12000, S-12001, S-21900).](image4)

![Figure 9.21 Excitation (dashed line) and emission (solid line) spectra of the SYPRO Ruby protein gel and blot stains (S-11791, S-12000, S-12001, S-21900).](image5)

![Figure 9.22 Excitation (dashed line) and emission (solid line) spectra of the SYPRO Ruby protein gel and blot stains (S-11791, S-12000, S-12001, S-21900).](image6)
SYPRO Orange and SYPRO Red protein gel stains are suitable for use with many laser-based scanning instruments.\(^1\)

- **Chemical stability.** The SYPRO Orange and SYPRO Red gel stains are chemically stable; fluorescence of the stained gel is stable for several days, and staining solutions can be stored for months.

- **Economy.** The SYPRO Orange and SYPRO Red gel stains are not only less expensive than silver-staining kits but faster and less laborious to use. Additionally, use of the SYPRO Orange or SYPRO Red dyes avoids the costs of purchase and disposal of large amounts of organic solvents that are required for Coomassie brilliant blue staining. Significant discounts are available on multiple-unit purchases of all of the SYPRO dyes for high-throughput research applications.

The SYPRO Orange and SYPRO Red stains have very similar staining properties, though we have observed that proteins stained with the SYPRO Orange dye are slightly brighter, whereas gels stained with the SYPRO Red dye tend to have lower background fluorescence. For maximum sensitivity with UV transilluminators, we recommend documenting the signal using Polaroid 667 black-and-white print film and the SYPRO photographic filter (S-6656, Figure 24.51). For maximum sensitivity with laser-based scanners, we recommend matching the appropriate SYPRO dye with the excitation light source of the instrument. For instance, SYPRO Orange protein gel stain is most suitable for gel scanners that employ argon-ion lasers with output at 488 nm, whereas SYPRO Red protein gel stain is best matched to laser-based scanning instruments that employ Nd:YAG lasers with output at 532 nm. Interestingly, the SYPRO Red dye is also compatible with scanners using excitation by the 633 nm spectral line of the He–Ne laser. The SYPRO Red protein gel stain has been used as a prestain for protein analysis in an automated ultrathin-layer gel electrophoretic technique.\(^29,30\) The SYPRO Orange protein gel stain has been used for protein sizing on a microchip\(^31\) and for analyzing the kinetics of isothermal protein denaturation.\(^32\)

The SYPRO Orange and SYPRO Red protein gel stains are compatible with SDS or urea/SDS gels. Staining native proteins in gels in the absence of SDS results in more protein-to-protein variation and lower sensitivity than staining SDS-denatured proteins, due to variations in hydrophobicity of the target polypeptides. However, sensitivity of SYPRO dye staining in native gels can be improved if gels are soaked in 0.05% SDS solution after electrophoresis but prior to staining. For optimal staining of proteins in 2-D gels and IEF gels, we recommend the SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900; see above).

Because the SYPRO Orange and SYPRO Red dyes do not covalently bind to proteins, stained proteins can be subsequently analyzed by microsequencing\(^24\) or mass spectrometry.\(^4,33\) However, these dyes are not recommended for staining gels prior to blotting, as there is a significant loss of sensitivity when proteins are stained with the SYPRO Orange or SYPRO Red dyes in typical Western blotting buffers. For maximum sensitivity and ease of use in staining proteins on blots, we recommend the use of SYPRO Tangerine protein gel stain (S-12010, see below) to stain proteins on the gel before blotting, or SYPRO Ruby or SYPRO Rose Plus protein blot stains (S-11791, S-12011; see below) for staining proteins on nitrocellulose or PVDF membranes after blotting.

**Figure 9.20** SYPRO Ruby protein gel stain is available in 200 mL (S-12001), 1 L (S-12000) or 5 L (S-21900) sizes.

**Figure 9.21** Comparison of the sensitivity achieved with SYPRO Orange, SYPRO Red, silver and Coomassie brilliant blue stains. Identical SDS-polyacrylamide gels were stained with A) SYPRO Orange protein gel stain (S-6650, S-6651), B) SYPRO Red protein gel stain (S-6653, S-6654), C) silver stain and D) Coomassie brilliant blue stain, according to standard protocols. The SYPRO Orange and SYPRO Red dye–stained gels were photographed using 300 nm transillumination, a SYPRO photographic filter (S-6656) and Polaroid 667 black-and-white print film. The silver- and Coomassie brilliant blue–stained gels were photographed with transmitted white light and Polaroid 667 black-and-white print film; no photographic filter was used to photograph these gels.

**Generous discounts are available for multiple-unit purchases of all of our SYPRO protein gel and blot stains. Contact us at order@probes.com.**
Photography of Gels and Blots

Photography is essential to obtain the maximum sensitivity for detection of proteins and nucleic acids in gels and blots. In most cases, an optical filter is required to exclude the exciting light and properly select the stain’s luminescence wavelengths. Gelatin optical filters, such as those described in Section 24.3, are low-cost solutions to photography of gels and blots.

The patented SYPRO Orange and SYPRO Red protein gel stains are available as 500 µL stock solutions in dimethylsulfoxide (DMSO), either in a single vial (S-6650, S-6653) or specially packaged as a set of 10 vials, each containing 50 µL (S-6651, S-6654). The reagents are supplied as 5000-fold concentrates; thus, 500 µL of either stain yields 2.5 L of staining solution. Significant discounts are available on multiple-unit purchases of all of the SYPRO dyes for high-throughput research applications. Photography of proteins in gels, which is essential for obtaining the maximum sensitivity, requires use of the SYPRO photographic filter (S-6656).

SYPRO Tangerine Protein Gel Stain: Sensitive Protein Staining without Fixation for Electrophoresis, Zymography and Classroom Use

Our SYPRO Tangerine protein gel stain \(^2\text{,}^7\) (S-12010) stains proteins in gels without the need for either acids or organic solvents and thus serves as an alternative to conventional protein stains that fix proteins in the gel.\(^2\) Whereas the SYPRO Orange and SYPRO Red stains require a staining solution containing acetic acid for maximum performance, staining with the SYPRO Tangerine protein gel stain is possible in almost any buffer that contains NaCl. Because proteins are not fixed during the staining procedure, they can be readily eluted from gels, used for zymography (in-gel enzyme activity assays, Figure 9.24) or analyzed by mass spectrometry.\(^2\) The SYPRO Tangerine stain can also be used to stain gels before transferring the proteins to nitrocellulose or PVDF membranes for immunostaining (Western blotting).\(^3\) Like the SYPRO Orange and SYPRO Red protein gel stains, the SYPRO Tangerine protein gel stain shows high sensitivity (down to \(~4\) ng/band) and a broad linear quantitation range (Figure 9.25). Environmentally friendly and easy to use, the SYPRO Tangerine protein gel stain is also ideal for use in educational settings, especially when used with UV-free blue-light transilluminators.

The SYPRO Tangerine stain is compatible with conventional SDS-polyacrylamide gel electrophoresis, but it is not recommended for 2-D or IEF gels. Stained proteins can be visualized using a UV transilluminator, a blue-light transilluminator or a laser scanner. Photography of stained gels using Polaroid 667 black-and-white print film requires use of the SYPRO photographic filter (S-6656) for optimal sensitivity.

The SYPRO Tangerine protein gel stain \(^2\text{,}^7\) (S-12010) is available as 500 µL of a 5000-fold concentrate in dimethylsulfoxide (DMSO), an amount sufficient to stain about 100 minigels. Significant discounts are available on multiple unit purchases.

SYPRO Protein Gel Stain Starter Kit

Our SYPRO Orange, SYPRO Red and SYPRO Tangerine protein gel stains are available in a SYPRO Protein Gel Stain Starter Kit (S-12012, Figure 9.26) for first-time users. Each kit includes:

- 50 µL of SYPRO Orange protein gel stain, sufficient for 5–20 minigels
- 50 µL of SYPRO Red protein gel stain, sufficient for 5–20 minigels
- 50 µL of SYPRO Tangerine protein gel stain, sufficient for 5–20 minigels
- A SYPRO protein gel stain photographic filter
- Detailed protocols

Protein Molecular Weight Standards

Molecular Probes offers a protein mixture for use as molecular weight markers in SDS-polyacrylamide gel electrophoresis (Figure 9.27). This broad-range marker mixture (P-6649) contains a balanced formulation of eleven polypeptides with molecular weights from 6500 to 205,000 daltons. These protein molecular weight standards give rise to sharp, well-separated bands when the gel is stained with any of our protein gel or blot stains. The mixture provides amounts of marker protein sufficient for loading about 200 gel lanes.

Fluorescent and Luminescent Total Protein Blot Stains

To characterize specific proteins in complex mixtures, proteins are frequently separated by electrophoresis, then blotted onto nitrocellulose or PVDF (poly(vinylidene difluoride)) membranes (blots) for immunostaining \(^{34}\) (referred to as Western blotting), glyco-
protein staining, sequencing or mass spectrometry. Universal protein stains provide valuable information about the protein samples on blots, making it possible to assess the efficiency of protein transfer to the blot, detect contaminating proteins in the sample and compare the sample with molecular weight standards. For blots of 2-D gels, staining of the entire protein profile makes it easier to localize a protein to a particular spot in the complex protein pattern. The superior properties of our fluorescent and luminescent protein stains, compared to conventional colorimetric stains, makes it possible to quickly and easily obtain this information without running duplicate gels. Combining our luminescent and fluorescent protein staining technology with our fluorescent reagents for specific protein detection, which are described in detail in Section 9.4, creates the capability of multiparameter staining, which we term Multiplexed Proteomics technology.

**SYPRO Ruby Protein Blot Stain: A Versatile Blot Stain**

The SYPRO Ruby protein blot stain \(^{7,13,35,36}\) (Table 9.4) provides fast and highly sensitive detection of proteins blotted onto membranes, making it easy to assess the efficiency of protein transfer to the blot and to determine if lanes are loaded equally \(^{37}\) (Figure 9.28). Because the stain does not covalently alter the proteins, it can be used to locate proteins on blots before sequencing or mass spectrometry.\(^{3,35}\) It can also be used before chromogenic, fluorogenic or chemiluminescent immunostaining procedures to locate molecular weight markers and visualize the total protein profile in the sample.\(^{35}\) Because the stain does not interfere with subsequent identification techniques, there is no need to run duplicate gels, vastly simplifying the comparison of total protein and target protein on Western blots. The SYPRO Ruby protein blot stain for general protein detection is also compatible with our Pro-Q Emerald glycoprotein blot stains for glycoproteins \(^{38}\) (Section 9.4, Figure 9.58). The superior properties of the SYPRO Ruby protein blot stain, as compared to conventional protein blot stains, make it possible to routinely stain blots for total protein before continuing with specific protein detection techniques.

The SYPRO Ruby protein blot stain \(^{7,13,21,35,36}\) (S-11791) combines the following superior staining characteristics:

- **High sensitivity.** SYPRO Ruby protein blot stain can detect as little as 0.25–1 ng protein/mm\(^2\) (~2–8 ng/band) blotted onto PVDF or nitrocellulose membranes after only 15 minutes of staining \(^{35}\) (Figure 9.28). This sensitivity on blots is far better than that of colorimetric stains, such as Ponceau S, amido black or Coomassie brilliant blue, and rivals the best colloidal gold blot-staining techniques (Figure 9.29).

- **Rapid total protein staining procedure.** The SYPRO Ruby protein blot-staining protocol takes less than an hour — including fixation and wash steps — and maximum sensitivity is achieved after only 15 minutes of dye staining, even for some peptides as small as seven amino acids. In contrast, gold or silver staining procedures may require overnight incubations to achieve maximum sensitivity and usually include extensive wash procedures that must be carefully timed.

**Table 9.4** Summary of fluorescent and luminescent protein blot stains.

<table>
<thead>
<tr>
<th>Dye Name</th>
<th>Ex/Em *</th>
<th>Major Applications</th>
<th>Features</th>
</tr>
</thead>
</table>
| SYPRO Ruby protein blot stain | 280, 450/618  | Mass spectrometry, microsequencing, counter-stain for blot-based detection techniques (nitrocellulose or PVDF membranes) | • Highest sensitivity (1–2 ng/band; comparable to colloidal gold staining)  
• Reversible                           |
| SYPRO Rose Plus protein blot stain | 350/610      | Mass spectrometry, microsequencing, counter-stain for blot-based detection techniques (nitrocellulose or PVDF membranes), reversible staining of proteins on surfaces | • Highest sensitivity (1–2 ng/band; comparable to colloidal gold staining)  
• Readily reversible                      |
| BODIPY FL-X †                 | 365, 505/575  | Counterstain for blot-based detection techniques (PVDF membranes)                    | • Very good sensitivity (4–8 ng/band)  
• Permanent, covalent staining for multicolor techniques |
| BODIPY TR-X ‡                 | 300, 590/615  | Counterstain for blot-based detection techniques (PVDF membranes)                    | • Very good sensitivity (4–8 ng/band)  
• Permanent, covalent staining for multicolor techniques |

* Excitation (Ex) and emission (Em) maxima, in nm. For maximum sensitivity, use excitation sources and optical filters matched to these values. † Available in DyeChrome Western Blot Stain Kits #1, #2 and #3 (D-21881, D-21882, D-21883; see Section 9.4). ‡ Available in DyeChrome Western Blot Stain Kits #4, #5 and #6 (D-21884, D-21885, D-21886; see Section 9.4).
The SYPRO Tangerine stain is the best choice for staining proteins without fixation.

- **Compatibility with Western blot protocols.** Staining total protein on the blot eliminates guesswork about transfer efficiency and removes the need to run two gels for comparison of total and target protein. The SYPRO Ruby protein blot stain is gentle and, unlike colorimetric or colloidal gold blot stains, does not interfere with subsequent colorimetric or chemiluminescent immunodetection of proteins on Western blots (Figure 9.36, Figure 9.45). The SYPRO Ruby protein blot stain is available as a component in four Pro-Q Glycoprotein Blot Stain Kits and four Pro-Q Western Blot Stain Kits (Table 9.5). These products are described in detail in Section 9.4.

- **Compatibility with standard microsequencing and mass spectrometry protocols.** Whereas colloidal gold, Coomassie brilliant blue and amido black staining can interfere with post-staining analysis, SYPRO Ruby protein blot stain binds noncovalently to proteins and is thus fully compatible with Edman sequencing or mass spectrometry.

- **Easily visualized signal.** The SYPRO Ruby protein blot stain comprises ruthenium complexes with an organic chelator. Because the ruthenium complex has dual-excitation maxima (Figure 9.30), the dye exhibits luminescence upon excitation with either UV or visible light. This property makes it possible to visualize the luminescence with many types of instruments, including UV epi-illumination sources, UV or blue-light transilluminators and a variety of laser-based scanning instruments, including those with excitation light at 450 nm, 473 nm, 488 nm or 532 nm. The red luminescence of the ruthenium complex has a peak at ~618 nm that is well separated from these excitation peaks, minimizing the amount of background signal seen from the excitation source. The staining signal can be documented using Polaroid 667 black-and-white print film and a SYPRO photographic filter (S-6656), using a CCD-based imaging system equipped with a 600 nm bandpass or 490 nm longpass filter, or by using the appropriate filter set and software in a laser-based scanner. The SYPRO Ruby protein blot stain has exceptional photostability, allowing long exposure times for maximum sensitivity.

The patented SYPRO Ruby protein blot stain (S-11791) is supplied as a 1x staining solution, sufficient for staining ~1600 cm² of blotting membrane and is accompanied by a detailed protocol for its use.

**SYPRO Rose Plus Protein Blot Stain: A Readily Reversible Protein Blot Stain**

Our patented SYPRO Rose Plus protein blot stain (S-12011) has the same high sensitivity detection capability as the SYPRO Ruby blot stain — about 0.25–1 ng protein/mm² (~2–8 ng/band) — and is fully compatible with subsequent immunostaining, lectin staining, mass spectrometry and Edman sequencing. However, unlike the SYPRO Ruby dye, the SYPRO Rose Plus dye produces protein staining that can be completely reversed by washing the blot. Because the staining can be so easily reversed, SYPRO Rose Plus protein blot stain may be useful for the temporary detection of proteins on other surfaces, like protein arrays, where it would be useful for signal normalization or quality control. It has been used to detect fingerprints on surfaces (Figure 9.31) and may be useful for detecting cells and proteins on contact lenses, electronic components and other surfaces for quality control purposes.

The SYPRO Rose Plus protein blot stain contains europium as the luminophore. The stain has an excitation maximum at ~350 nm and a narrow emission peak at ~610 nm (Figure 9.32). Stained proteins can be visualized using UV epi-illumination; the excitation characteristics of the SYPRO Rose Plus protein blot stain preclude it from being visualized using visible-wavelength excitation sources. Like the SYPRO Ruby protein blot stain, the SYPRO Rose Plus protein blot stain has exceptional photostability. In addition, the europium luminescence has a very long emission lifetime (20–50 µsec), which should allow time-resolved luminescence measurements that would greatly minimize background fluorescence.

The SYPRO Rose Plus protein blot stain (S-12011) is provided as a kit containing:

- SYPRO Rose Plus blot stain solution
- SYPRO Rose blot wash solution
- SYPRO Rose blot destain solution
- A detailed protocol

The quantities of reagents are sufficient to stain ~1600 cm² of blotting membrane.
Reactive Fluorescent Dyes for Permanent Protein Blot Staining

Our patented BODIPY reactive dyes in Section 1.4 label amine groups (predominantly lysine residues) on proteins, and we have found two of them to be particularly effective general stains for proteins on PVDF membranes. This unique method of staining for total proteins on blots with the reactive BODIPY dyes has a 30-fold linear dynamic range (Figure 9.41), although the absolute intensity between proteins may vary somewhat with the nature of the protein. Reactive BODIPY dye–based staining is rapid, simple and highly sensitive, permitting detection of as little as 4 ng of a protein per band in about an hour. Because the reactive dyes form a covalent bond with the protein, the staining is permanent and lasts through any subsequent conventional blot manipulations. The covalent modification appears to minimally interfere with subsequent immunostaining, as we have successfully performed simultaneous two-color labeling with reactive BODIPY dyes and either fluorogenic immunostains or fluorogenic glycoprotein stains and found both stains to be visible at the same time on the same blot (Figure 9.39, Figure 9.40). Simultaneous dual labeling of a sample enormously simplifies localization of a specific protein with respect to other proteins in the sample, particularly on electroblots of 2-D gels, which are difficult to align when used as pairs of gels.

The optimal BODIPY dyes and procedures for use with our fluorogenic Western blot detection reagents are included in our DyeChrome Western Blot Stain Kits (Section 9.4, Table 9.5). The green-fluorescent BODIPY FL-X dye is used in combination with DDAO phosphate (Figure 9.40, Figure 9.43), which produces a red-fluorescent product in the presence of alkaline phosphatase; the red-fluorescent BODIPY TR-X dye is used in combination with ELF 39 phosphate (Figure 9.39, Figure 9.44), which produces a green-fluorescent product in the presence of alkaline phosphatase. The fluorescent staining can be visualized using UV illumination or a laser-based scanner. The stains can be documented simultaneously using color photography or — using the appropriate filters, such as those in the DyeChrome Red/Green Photographic Filter Set (D-24771; Section 24.4; Figure 24.52, Figure 24.53) — can be documented separately. Note that because reaction of the dye covalently modifies the protein at random locations, staining by the amine-reactive BODIPY dye may complicate or preclude subsequent analysis by mass spectrometry or microsequencing.

Our DyeChrome Double Western Blot Stain Kit (D-21887, Section 9.4) uses another fluorescent reactive dye, MDPF (2-methoxy-2,4-diphenyl-3(2H)-furanone), for staining of the total protein profile on PVDF membranes. This blue-fluorescent dye is visible using UV epi-illumination and can be used with two (or possibly more) different fluorogenic Western blot stains. In the DyeChrome Double Western Blot Stain Kit, MDPF is used together with DDAO phosphate, which produces the red-fluorescent DDAO dye (Figure 10.6, Figure 10.11) in the presence of alkaline phosphatase, and our proprietary Amplex Gold reagent, which produces a yellow-fluorescent compound in the presence of horseradish peroxidase, for detection of two different specific proteins and the total protein profile, all on the same blot (Section 9.4, Figure 9.48).

Detection of Proteins on Protein Microarrays

We have found that our protein blot stains perform particularly well for staining proteins on PVDF microarrays for quality control and normalization purposes. SYPRO Ruby protein blot stain (S-11791) shows good sensitivity on protein microarrays (Figure 9.33) and should be very useful for staining proteins before exposing the microarray to the sample. The stain washes off of the PVDF membrane very easily under conditions used in typical Western blot blocking buffers. BODIPY FL SE (D-2184) shows even greater sensitivity in this application (Figure 9.33) and should be useful for quality control or as an internal normalization standard.

Protein Detection in Capillary Electrophoresis

Capillary electrophoresis (CE) is an exceptionally powerful tool for the resolution of biomolecules. Fluorescent detection of proteins that are separated by capillary electrophoresis can occur either during the run — the more common procedure — or subsequent to the separation on isolated fractions. When detected during the electrophoretic separation, the protein is either derivatized with a fluorescent reagent prior to the separa-
or labeled with a fluorescent dye that is incorporated into the running medium. In general, the same reagents may be useful for fluorometric detection of peptides and proteins that are separated by high-performance liquid chromatography (HPLC).

**Use of SYPRO Dyes for Capillary Electrophoresis**

SDS–capillary gel electrophoresis (SDS–CGE) separates proteins based on principles similar to those of standard slab-gel electrophoresis, but with the advantages of faster run times, higher resolution and greater sensitivity. The use of on-line detection by laser-induced fluorescence (LIF) increases the sensitivity several orders of magnitude over UV detection, eliminates the time spent staining and photographing the gel and allows for the possibility of automated sample processing. SDS-CGE analyzed by LIF is already being widely used for the separation and identification of DNA fragments and has increased the efficiency of genomics, DNA typing and forensics laboratories.\(^{42,43}\) SDS-CGE promises to be just as useful for proteomics laboratories and other laboratories that require characterization of a large number of protein samples.

For SDS-CGE of protein samples, the amine groups of lysine residues and the N-terminus of the proteins are typically derivatized with a fluorescent or fluorogenic dye such as the ATTO-TAG CBQCA\(^ {44,45}\) (A-2333, A-6222) or ATTO-TAG FQ\(^ {46}\) (A-2334, A-10192) reagents before separation in the capillary. The derivatized proteins are then coated with SDS and travel through the capillary gel towards the positive electrode based on their size, with smaller proteins traveling faster. The derivatized proteins are detected by fluorescence emitted as they pass a laser that excites the fluorophores. One disadvantage of this technique is that the proteins generally contain multiple amine groups, each of which can react with the derivatization reagent. Typically, only a few of the amine groups on each protein molecule react, and the result is an enormous number of different...
derivatives, creating broad peaks that may be difficult to correlate with the original protein’s structure or abundance. In addition, variations between runs make it difficult to reproducibly estimate molecular weights. In contrast, use of SYPRO Red protein gel stain (S-6653, S-6654) to precast SDS-coated proteins allows more accurate determination of molecular weights because the proteins are relatively uniformly coated with SDS and the dye. This method leads to molecular weight determinations similar in accuracy to that achieved with polyacrylamide slab gels, with a limit of detection estimated to exceed the detection limit of silver staining in slab gels.

Use of SYTO, SYBR and NanoOrange Dyes for Capillary Electrophoresis

Our SYTO and SYBR dyes — which are extremely useful stains for nucleic acids (Chapter 8) — are essentially nonfluorescent in aqueous medium unless they are bound to nucleic acids. However, we have found that these same dyes may become highly fluorescent once bound to lipid-complexed proteins. Although we are not aware of any published description of the use of these dyes for detecting proteins in capillary electrophoresis, we anticipate that they can be used in the same manner as the SYPRO dyes for these applications. The broad spectral range of available SYTO (Table 8.3) and SYBR dyes (Table 8.1) should permit their use with a wide variety of excitation sources. Several of the SYTO and SYBR dyes also stain proteins in SDS-polyacrylamide gels, but usually with lower sensitivity than do the SYPRO dyes. The NanoOrange reagent (in kit N-6666, Section 9.2) is reported to be an optimal reagent for detecting proteins that have been separated by microchip capillary electrophoresis.

Derivatization Reagents for Proteins

Several of the same reagents that were described in Section 9.2 for protein quantitation in solution are also useful for peptide and protein derivatization, either prior to or following separation by capillary electrophoresis. However, chemical derivatization prior to separation is likely to change the electronic charge and always changes the mass of the protein. Furthermore, incomplete derivatization of amines or thiols on the protein can lead to a pure protein resolving into multiple species in the electrophoretogram.

In an improved procedure for fluorescent analysis of peptides by capillary electrophoresis, Zhou and colleagues modified all α- and ε-amino groups of the peptide with phenyl isothiocyanate. Following one cycle of Edman degradation, the single free α-amino group was modified with fluorescent reagents to give a homogeneous, dye-labeled peptide.

The preferred reagents for derivatizing amine residues in proteins either prior to or following electrophoretic separation are those that are essentially nonfluorescent until reacted with the protein. Derivatization reagents that react with thiols or other functional groups have also been used. These preferred reagents include:

- ATTO-TAG CBQCA, which is available in our ATTO-TAG CBQCA Amine-Derivatization Kit (A-2333) and as a standalone reagent (A-6222). ATTO-TAG CBQCA reacts with phosphoserine residue.

Figure 9.33 Protein detection on microarrays using BODIPY FL-X SE or SYPRO Ruby protein blot stain. Bovine serum albumin was arrayed onto a PVDF membrane using a Packard BioScience PiezoTip dispenser. The array contains 72 spots with 12 dilutions of the dye, in replicates of 6, ranging from 666–0.325 pg per spot. The proteins were stained with either BODIPY FL-X SE (D-6116, left) in sodium borate buffer, pH 9.5, or with SYPRO Ruby protein blot stain (S-11791). Arrays were imaged on a ScanArray 5000XL microarray analysis system (Packard BioScience) and pseudocolored such that the different colors indicate different signal intensities. The sensitivity limit was 1.3–2.6 pg of protein in a 175 µm spot for the BODIPY FL-X SE detection technique and 104 pg of protein per 175 µm spot for the SYPRO Ruby protein blot stain technique.

Our new Zenon technology (Section 7.2) represents a major opportunity for selective detection of proteins on microarrays. Zenon labeling of antibodies is easily miniaturized and automated. See our special Zenon Web site (www.probes.com/zenon) for further information.

Figure 9.34 Selective detection of phosphoserine residues in proteins via derivatization by 1,2-ethanedithiol, followed by 6-iodoacetamidofluorescein (I-15).
primary amines to form highly fluorescent isoindoles, and has been extensively used for the derivatization of amino acids, peptides, and carbohydrates prior to capillary electrophoretic separation. ATTO-TAG CBQCA has been used to derivatize a fusion protein expressed in the bacterium Escherichia coli before purification by capillary zone electrophoresis. After purification, the fluorescent isoindole can be removed by acid treatment to allow sequencing in the bacterium CBQCA has been used to derivatize a fusion protein expressed prior to capillary electrophoretic separation. A TTO-TAG FQ (3-(2-furoyl)quinoline-2-carboxaldehyde), the fluorescent reagent that rapidly reacts with amines to give a fluorescent product, has been used for solution quantitation of proteins and peptides (Section 9.2). It is also useful as a peptide and protein detection reagent for capillary electrophoresis. Use of fluorescamine to derivatize a standard protein of known molecular weight together with use of the ATTO-TAG FQ reagent to derivatize the sample protein allows the sample to be run simultaneously with the standard, improving the accuracy of molecular weight determination.

- Dialdehydes OPA and NDA (P-2331, N-1138), which react with amines in the presence of a nucleophile (Figure 1.103, Figure 1.104) to give fluorescent products. These inexpensive reagents have been used for capillary electrophoresis of peptides and proteins.
- Other amine-reactive reagents. Chapter 1 describes a variety of other amine-reactive reagents, including our numerous succinimidyl esters, isothiocyanates and sulfonyl chlorides, that have been used or may be useful for peptide and protein detection in capillary electrophoresis, including dansyl chloride (D-21), NBD chloride (Fluoropure Grade, C-20260), NBD fluoride (F-486), FITC (P-2336) and other common reagents described in Section 1.8.
- Reactive reagents for other groups on proteins. Thiol-reactive probes such as maleimides and iodoacetamides can be used for the selective detection of natural or engineered proteins that contain a free thiol group (cysteine). Most of the fluorescent derivatization reagents for thiols (Chapter 2) could potentially be used for detection of thiolated proteins in capillary electrophoresis. Thiol-reactive reagents that are essentially nonfluorescent until conjugated to thiols, such as the coumarin maleimides CPM and DACM (D-346, D-10251; Section 2.3), monobromobimane (M-1378; Fluoropure Grade, M-20381; Section 2.3) and N-(1-pyrene)maleimide (P-28, Section 2.3), should work well in this application. A particularly unique derivatization scheme using 6-idoacetamidofluorescein (I-15, Section 2.2) has been applied to selective detection of peptides and proteins containing phosphoserine residues by capillary electrophoresis (Figure 9.34). Although the original procedure used 6-idoacetamidofluorescein, almost any of the thiol-reactive reagents in Chapter 2 may be useful for this method. To identify glycoproteins in capillary electrophoresis, reagents derived from hydrazide derivatives such as those described in Section 3.2 may be useful for labeling the hydroxyl groups after oxidation to aldehydes. Dansyl hydrazine (D-100) to give fluorescent products. These inexpensive reagents have been used for capillary electrophoresis of peptides and proteins.

### References

# Data Table — 9.3 Detection of the Total Protein Profile in Gels, on Blots and in Capillary Electrophoresis

<table>
<thead>
<tr>
<th>Cat #</th>
<th>MW</th>
<th>Storage</th>
<th>Soluble</th>
<th>Abs</th>
<th>EC</th>
<th>Em</th>
<th>Solvent</th>
<th>Notes</th>
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<td>A-2333</td>
<td>305.29</td>
<td>F.D.L.</td>
<td>MeOH</td>
<td>465</td>
<td>ND</td>
<td>560</td>
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<tr>
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<td>ND</td>
<td>591</td>
<td>MeOH</td>
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<td>N-1138</td>
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<td>DMF, MeCN</td>
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<td>EtOH</td>
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<td>L</td>
<td>see Notes</td>
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<td>10, 11, 12</td>
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</table>

For definitions of the contents of this data table, see “How to Use This Book” on page viii.

**Notes**
1. Spectral data are for the reaction product with glycine in the presence of cyanide. Unreacted reagent in MeOH: Abs = 254 nm (EC = 46,000 cm·M⁻¹), nonfluorescent.
2. ND = not determined.
3. Solubility in methanol is improved by addition of base (e.g., 1–5% (v/v) 0.2 M KOH).
4. Data represent the reactive dye component of this labeling kit.
5. Spectral data are for the reaction product with glycine in the presence of cyanide. Unreacted reagent in MeOH: Abs = 282 nm (EC = 21,000 cm·M⁻¹), nonfluorescent.
6. Fluorescamine spectra are for reaction product with butylamine. Fluorescence quantum yield/lifetime of adduct in EtOH are 0.23 and 7.5 nanoseconds, respectively (Arch Biochem Biophys 163, 390 (1974)). Unreacted reagent in MeOH: Abs = 279 nm (EC = 5500 cm·M⁻¹), Em = 330 nm.
7. This product is specified to equal or exceed 98% analytical purity by HPLC.
8. Spectral data are for the reaction product with glycine in the presence of cyanide, measured in pH 7.0 buffer/MeCN (40:60) (Anal Chem 59, 1102 (1987)). Unreacted reagent in MeOH: Abs = 257 nm (EC = 1000 cm·M⁻¹).
9. Spectral data are for the reaction product of P-2331 with alanine and 2-mercaptoethanol. The spectra and the stability of the adduct depend on the amine and thiol reactants (Biochim Biophys Acta 576, 440 (1979)). Unreacted reagent in H₂O: Abs = 257 nm (EC = 1000 cm·M⁻¹).
10. This product is supplied as a ready-made staining solution.
11. The active ingredient of this product is an organometallic complex with MW <1300. The exact MW value and extinction coefficient of the complex are proprietary.
12. SYPRO Ruby protein gel stain also has an absorption peak at 278 nm with about fourfold higher EC than the 462 nm peak.

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# Product List — 9.3 Detection of the Total Protein Profile in Gels, on Blots and in Capillary Electrophoresis

<table>
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<tr>
<th>Cat #</th>
<th>Product Name</th>
<th>Unit Size</th>
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<tr>
<td>A-2333</td>
<td>ATTO-TAG™ CBQCA Amine-Derivatization Kit</td>
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<td>A-6222</td>
<td>ATTO-TAG™ CBQCA derivatization reagent (CBQCA; 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde)</td>
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<td>A-2334</td>
<td>ATTO-TAG™ FQ Amine-Derivatization Kit</td>
<td>1 kit</td>
</tr>
<tr>
<td>A-10192</td>
<td>ATTO-TAG™ FQ derivatization reagent (FQ; 3-(2-furoyl)quinoline-2-carboxaldehyde)</td>
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<td>F-2332</td>
<td>fluorescamine</td>
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<tr>
<td>F-20261</td>
<td>fluorescamine &quot;FluoPure™ grade&quot;</td>
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<td>naphthalene-2,3-dicarboxaldehyde (NDA)</td>
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<td>α-phthalaldehyde (OPA) “high purity”</td>
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<td>protein molecular weight standards “broad range” *200 gel lanes</td>
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Molecular Probes has an extensive patent portfolio that includes the SYPRO dyes and their commercial use for protein detection. Licenses are available by contacting busdev@probes.com. Generous discounts are available for multiple-unit purchases of all of our SYPRO protein gel and blot stains. Contact us at order@probes.com.