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Protein Detection in Gels Using Fixation *UNIT 10.5*

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Abstract

The most commonly used types of gels for separating proteins are SDS gels either in a 1-D format or as the second dimension of various 2-D separations, and the most common methods of visualizing proteins in these gels use protein binding dyes after fixing the proteins in the gel matrix. In recent years, there has been a continuing trend away from preparing staining solutions in the laboratory to using commercially available kits, which are convenient, save time, have defined shelf lives, and may provide greater reproducibility than stains formulated in research laboratories. For common types of stains such as Coomassie and silver stains, wide ranges of staining kits are now available that may enhance specific features such as sensitivity, staining speed, or environmental compatibility. Associated differences in stain formulations are often proprietary and performance relative to alternative stains may not be readily predictable from product descriptions. For example, different commercial colloidal Coomassie stains may vary substantially in sensitivity, background, ease of use, and consistency of staining across different proteins. Other potential limitations of commercial kits include the “black box” nature of proprietary stains and cost, especially for laboratories using large volumes of stains. In general, when using commercial kits, satisfactory results can be readily obtained by following the manufacturer’s protocols. This unit reviews commonly used fixation-based stains and provides a number of manual formulations with staining protocols for those who prefer such staining methods.

Keywords

SDS gel stains; Coomassie stains; SYPRO Ruby; Silver stains; MS compatible

Detection of proteins in polyacrylamide gels is essential for evaluating the composition and changes in composition of biological samples for diverse applications. In addition, 1-D gels have become a preferred method for microscale isolation of small amounts of proteins for identification using internal cleavage with proteases followed by mass spectrometry analysis

Key References

Miller et al., 2006. See above.

Overview of protein stains and gel-imaging devices.

Patton, 2000. See above.

Describes fluorescent stains.

Rabilloud, T. 1992. A comparison between low background silver diammine and silver nitrate protein stains. *Electrophoresis* 13:429–439.

Compares many different silver staining methods.

Wilson, C. 1983. Staining of proteins on gels: Comparison of dyes and procedures. *Methods Enzymol*. 91:236–247.

Reviews different Coomassie blue staining procedures.

methods (Chapter 16). Such analyses may involve isolation of very complex samples such as a cell or tissue lysate, a purified organelle or protein complex, or a highly enriched single protein. This unit presents basic protocols for detection of proteins by Coomassie blue staining (see Basic Protocol 1), staining with SYPRO Ruby a preformulated, noncovalent fluorescent stain (see Basic Protocol 2), and silver staining (see Basic Protocol 3). All protocols involve fixing the protein within the gel. Alternate protocols for Basic Protocol 1 detail rapid colloidal Coomassie blue staining (Alternate Protocol 1), which is slightly less sensitive, and acid-based colloidal Coomassie blue staining (Alternate Protocol 2), which avoids the need for destaining. Basic Protocol 3 for silver staining is accompanied by three alternate protocols: nonammoniacal silver staining (Alternate Protocol 3), which uses more stable solutions; rapid silver staining (Alternate Protocol 4), which involves fixation with formaldehyde instead of glutaraldehyde; and the enhanced-background, two-stage method (Alternate Protocol 5), which employs fixation without cross-linking to enhance sensitivity and also includes an optional reduction step to reduce background staining. A Support Protocol describes methods to obtain images of gels for archiving and quantitative analyses.

The protocols in this unit and *UNIT 10.6* require proteins that have been previously separated by one- or two-dimensional electrophoresis (*UNITS 10.1–10.4*). Coomassie blue staining is relatively quick and inexpensive, whereas SYPRO Ruby and silver staining are considerably more expensive but are typically between 10 and 100 times more sensitive. When subsequent removal of intact proteins from the gel is desired, detection methods without fixation should be used (*UNIT 10.6*).

In recent years, the number of commercially available preformulated gel stains has steadily increased to the point where multiple variations of every common type of stain can be purchased. Commercial staining kits offer convenient alternatives to preparing gel-staining solutions in the laboratory. They generally save time, have defined shelf lives, and often provide greater quality control and reproducibility than stains formulated in different research laboratories. In many cases, staining kits have been modified by the manufacturers to enhance specific features such as sensitivity, staining speed, and environmental compatibility. These modifications and differences in stain formulations are usually proprietary; therefore, the effects of specific commercial products on different proteins and gel systems may vary and cannot be readily predicted from product descriptions. For example, not all commercial colloidal Coomassie stains will have the same sensitivity, background, and ease of use. Hence, to identify the best staining kit for a specific purpose, it may be desirable to evaluate different products based on the application of interest. There are particularly wide ranges of choice for silver stain kits due to the relative complexity of the basic method and the alternative chemistries that can be used. An overview of the features for Coomassie, silver and fluorescent staining methods is summarized in Table 10.5.1. Common types of preformulated stains in these three categories and their characteristics are summarized in Table 10.5.2–10.5.4.

One potential limitation of staining kits for some applications might be the “black box” nature of working with undefined proprietary stains. Another limitation may be their generally higher cost compared with staining solutions formulated on site, especially when only the costs of consumables are considered. However, preformulated stains can be more

economical when volumes of stain used are modest and labor costs are considered. Nonetheless, for those cases where manual formulation is preferred, stain preparation and use protocols are described here for conventional Coomassie R-250, G-250, and silver stains.

COOMASSIE BLUE R-250 STAINING

Coomassie brilliant blue R-250 binds nonspecifically to almost all proteins, which allows detection of protein bands in polyacrylamide gels. This stain is very popular because it is relatively rapid, simple, inexpensive, and stains most proteins with similar intensities. Its major limitation is that it is less sensitive that the other stains described in this unit. In this procedure, proteins separated on a polyacrylamide gel are simultaneously fixed and stained in the Coomassie blue staining solution, which turns the entire gel blue. Destaining eliminates the background while the protein bands retain the blue color. Gels can be photographed or dried to maintain a permanent record. Alternatively, the “wet” gel can be sealed in a plastic bag with a small amount of destaining solution and stored for many months at 4°C.

Materials

Coomassie blue R-250 staining solution (see recipe)

Polyacrylamide gel containing protein of interest (*UNITS 10.1–10.4*)

Destaining solution: 15% methanol/10% acetic acid (v/v; store up to 1 month at room temperature)

7% (v/v) acetic acid (optional)

Plastic or glass container with tight-fitting lid of size appropriate for gel

Platform shaker

Protein-based destaining material (optional): white 100% wool yarn, cellulose sponge, *or* Whatman 3MM filter paper

Resealable plastic bags

Gel dryer (optional)

NOTE: Only high-purity water such as Milli-Q-purified water or equivalent should be used throughout the protocol, unless otherwise specified.

1. Add ~10 gel volumes Coomassie blue R-250 staining solution to a plastic or glass container with lid.
2. Remove polyacrylamide gel from the electrophoresis unit and place in staining solution, then place container on a platform shaker and agitate gel slowly to prevent it from adhering to the container.

CAUTION: *Rapid agitation can result in gel breakage*.

The appropriate length of time to stain a gel depends on the gel thickness as well as the percentage of acrylamide. In general, gels ≤1 mm thick require only 30 min to 1 hr of staining, whereas gels ~1 mm thick require a minimum of 1 to 3 hr of staining. Also, the higher the percentage of acrylamide, the longer it will take to stain the gel. Gels can be left in staining solution overnight without adverse effects.

3. Remove staining solution and rinse the gel briefly with deionized water to remove excess stain.

   Do not reuse staining solution.

4. Add 5 to 10 gel volumes destaining solution and place the container with gel on shaker.

   For more efficient destaining and to reduce hazardous waste, a protein-based destaining material such as a piece of wool yarn (6 to 12 in. long), a cellulose sponge (1 in. × 1 in.), or Whatman 3MM filter paper (1 in. × 1 in.) can be added to a minimal amount of destaining solution to bind the dye as it is released from the gel. Alternatively, the destaining solution can be changed several times until the gel shows a clear background with blue protein bands.

   CAUTION: *Overdestaining can occur if the gel is left in destaining solution for a prolonged period (~24 hr) or if the dye-binding material is left with the gel in the destaining solution for more than 2 to 3 hr*.

5. For storage, place gel in a thermally sealed or resealable plastic bag with a small amount (~1 ml for large format gel or a few tenths of a ml for a mini-gel) destaining solution or 7% acetic acid.

   Gels can be stored sealed in plastic bags for several weeks at room temperature or for many months at 4°C.

6. For a permanent record, scan or photograph the gel (see Support Protocol), or dry the gel as follows. Place a wet piece of Whatman 3MM filter paper on a gel dryer and position the gel (rinsed with water) on top of the filter paper. Cover gel with a piece of plastic wrap and then add a second piece of wet filter paper. Dry 1 to 2 hr at ~80°C or according to gel dryer instructions. As an alternative gel drying method, use a porous membrane to sandwich the gel in a supporting frame. In this case, dry the gel on the countertop at room temperature overnight.

ALTERNATE

PROTOCOL 1

RAPID COOMASSIE BLUE G-250 STAINING—This protocol utilizes Coomassie brilliant blue G-250 in place of the Coomassie brilliant blue R-250 used in Basic Protocol 1. Also called xylene brilliant cyanin G, Coomassie brilliant blue G-250 (greenish hue) usually stains with a somewhat higher sensitivity than Coomassie brilliant blue R-250 (reddish hue).

The G-250 stain produces a brighter blue coloration than the deep blue of the R-250 stain. The G-250 dye is less soluble than R-250 and is used in a colloidal state rather than as a solution. Due to its lower solubility, G-250 preferentially binds to the protein bands and not to the polyacrylamide gel itself; therefore, little or no destaining is necessary. The procedure requires fixation of gels prior to staining. Protein staining then develops rapidly and can be observed easily throughout the staining solution. This procedure provides faster detection of proteins in gels than Basic Protocol 1.

Additional Materials (also see Basic Protocol 1): Isopropanol fixing solution: 25% isopropanol/10% acetic acid (v/v; store several months at room temperature)

Rapid Coomassie blue G-250 staining solution: 10% (v/v) acetic acid/0.006% (w/v) Coomassie brilliant blue G-250 (Bio-Rad; store several months at room temperature)

10% (v/v) acetic acid

1. To a plastic container with lid add ~10 gel volumes isopropanol fixing solution. Remove gel from the electrophoresis unit and place in fixing solution. Agitate container on a platform rocker or shaker for 10 to 15 min for 1-mm-thick gel or 30 to 60 min for 1.5-mm-thick gel.
2. Discard fixing solution and add 5 to 10 gel volumes rapid Coomassie blue G-250 staining solution. Agitate 30 min to 1 hr for 1-mm-thick gel or 2 to 3 hr for 1.5-mm-thick gel.

   Staining of protein bands becomes apparent within 5 to 15 min.
3. Remove staining solution after appropriate time. Add 5 to 10 gel volumes of 10% acetic acid and agitate slowly. Destain (1 to 2 hr) until a clear background with blue protein bands appears.
4. For storage, place gel in a resealable plastic bag at 4°C with a small amount of 10% acetic acid.
5. To scan or photograph gel, see Support Protocol. To dry gel, see Basic Protocol 1 (step 6).

ALTERNATE

PROTOCOL 2

ACID-BASED COOMASSIE BLUE G-250 STAINING—The acid-based Coomassie brilliant blue G-250 staining procedure fixes and stains the gels simultaneously and, unlike the first basic and alternate protocols, does not require a destaining solution. Color intensifies when the gels are placed in water after the staining period, and as little as 50 to 100 ng protein per band can be detected. Acid-based Coomassie blue G-250 which is also used in a colloidal state rather than a solution, staining has a sensitivity similar to that of the basic Coomassie blue R-250 staining. More heavily loaded protein bands can be detected within 30 min, but ~5 hr is needed to visualize light bands.

Additional Materials (also see Basic Protocol 1): Acid-based Coomassie blue G-250 staining solution (see recipe)

1. Remove polyacrylamide gel from the electrophoresis unit. If gel contains SDS, incubate in 5 to 10 gel volumes deionized water for a minimum of 5 to 15 min depending on gel thickness to allow SDS to diffuse out.

   SDS will precipitate in the staining solution and produce an opaque background.

2. Place gel into a container with ~100 ml acid-based Coomassie blue G-250 staining solution. Place gel on a platform shaker and agitate gently for 5 hr to overnight.

   Heavily loaded proteins will be visible within 30 min. However, greatest sensitivity is achieved by staining for at least 5 hr.

3. Decant staining solution and replace with deionized water.

   Even after the gel has been stained overnight, protein bands will not be completely resolved until the gel is placed into deionized water. Bands will appear bright blue against a clear background within 2 to 5 min.

4. For storage, place gel in 1 to 2 ml water in a resealable plastic bag at 4°C.

5. To scan or photograph gel, see Support Protocol. To dry gel, see Basic Protocol 1 (step 6).

BASIC

PROTOCOL 2

SYPRO RUBY STAINING—There are several commercially available fluorophores which interact with proteins in polyacrylamide gels through noncovalent mechanisms similar to Coomassie stains and that are more sensitive than Coomassie brilliant blue or colloidal Coomassie stains. These fluorescent stains, summarized in Table 10.5.3, have wider dynamic ranges than colorimetric stains. The most sensitive of these dyes, SYPRO Ruby, can be visualized using a standard 302-nm UV transilluminator or imaging system containing the appropriate excitation and emission filters (see Support Protocol). The maximal excitation and emission wavelengths are 450 and 610 nm, respectively. SYPRO Ruby does not interfere with common downstream analysis methods such as mass spectrometry, Edman sequencing, or immunodetection procedures. Depending upon the imaging method used, the sensitivity of this stain can be comparable to most silver staining methods. The protocol for SYPRO Ruby staining described below has been adapted from the method described by the manufacturer of the fluorophore(Thermo Fisher).

Materials: SDS 1- or 2-D polyacrylamide gels containing proteins of interest (*UNITS 10.1–10.4*)

Fixing solution: 40% (v/v) methanol/10% (v/v) acetic acid in H2O

SYPRO Ruby protein gel stain (Thermo Fisher)

Destain/wash solution: 2% (v/v) acetic acid

2% (w/v) glycerol

Plastic container with tight-fitting lid, of size appropriate for gel

Platform shaker

Additional reagents and equipment for acquiring gel images (see Support Protocol)

1. Remove polyacrylamide gel from the electrophoresis unit and place in a plastic container containing at least 20 gel volumes of 50% methanol/7% acetic acid. Cover with a tight-fitting lid and fix the gel for 30 min on a platform shaker with continuous, gentle agitation.

   Polypropylene or polycarbonate plastic containers are optimal because they absorb a minimal amount of the dye.

2. Remove fixing solution and repeat once more with 20 gel volumes of fresh fix solution for 30 min.

3. Remove fixing solution and incubate gel in 10 gel volumes of undiluted SYPRO Ruby stain for 3 hr to overnight with gentle agitation, protected from light.

   All reagents should be carefully removed by vacuum aspiration. The gel should be protected from light from this step forward, until an image has been obtained for archiving. The manufacturer does not recommend using less than 10 gel volumes of stain or reusing the stain because reduced staining sensitivity can result. However, if at least 10 gel volumes of stain are used and the stain is protected from light, the reduction in sensitivity is minor when the stain, which is quite expensive, is reused once. However, with further re-use of the stain, staining intensity will be more dramatically reduced and different proteins can be affected to a varying extent (Ahnert et al, 2004).

4. Transfer the gel to a clean container and wash the gel by immersing in 20 gel volumes of 2% acetic acid for 30 min to reduce background fluorescence and increase sensitivity.

   For convenience, the gel may be washed overnight without a loss in sensitivity when 2% acetic acid is used as the destain solution. If 10% methanol (or ethanol) is used, background fluorescence reduction is more rapid, but protein band intensities will begin to fade appreciably after about 30 min (exact optimal time depends on gel thickness).

5. Acquire an image of the gel for a permanent record (see Support Protocol).

   The stained gel can be stored for several days in 2% acetic acid in the dark at room temperature or for at least a week at 4°C with minimal reduction in signal intensity.

6. *Optional:* For longer-term storage, transfer the gel into a 2% glycerol solution, incubate for 30 min, then dry gel in a gel dryer.

BASIC

PROTOCOL 3

SILVER STAINING—Silver staining is about 50 to 100 times more sensitive than Coomassie blue staining; however, staining responses can vary greatly from protein to protein. After alcohol/acid fixation and treatment with silver nitrate, the gel is developed until a desired staining intensity is reached. Achieving reproducible staining intensity is very difficult because extent of staining is affected by the exact temperature and length of time in the developer as well as gel thickness. In addition, staining intensity can vary widely for different proteins. Therefore, estimation of protein amounts using silver staining is not advisable unless known quantities of the protein of interest are included on the same gel to calibrate staining intensity. Reagent volumes, reaction times, and temperature should be carefully controlled, and the very narrow linear detection range must be considered when silver staining is used to compare protein patterns on 2-D gels. The following protocol is applicable to 1-D and 2-DSDS-PAGE, and is based on the ExPASy SWISS-2D PAGE method, which has been optimized to give uniformly brown spots on a light background to facilitate image acquisition and quantitative comparisons. However, this method is not recommended for protein identification using mass spectrometry because the extensive chemical modification of proteins during the staining procedure, especially the chemical cross-linking of proteins by glutaraldehyde, greatly diminishes detection of unmodified peptides that can be identified in database searches of LC-MS/MS data.

Materials: Polyacrylamide gel containing proteins of interest (see *UNITS 10.1–10.4*; also see recipe)

Fixing solution A: 40% (v/v) ethanol/10% (v/v) acetic acid in water

Fixing solution B: 5% (v/v) ethanol/5% (v/v) acetic acid in water

0.5 M sodium acetate containing 1% (v/v) glutaraldehyde

0.05% (w/v) 2,7-napthalene disulfonic acid

Ammoniacal silver nitrate solution (see recipe)

Citric acid developing solution (see recipe)

Stop solution: 5% (w/v) Tris base containing 2% (v/v) acetic acid

Glass or plastic container with tight-fitting lid, of size appropriate for gel

Platform shaker

Resealable plastic bags

Additional reagents and equipment for acquiring gel images (see Support Protocol)

CAUTION: Ammoniacal silver nitrate solution becomes explosive on drying. Discard immediately after use by adding 1 M HCl to precipitate the silver as silver chloride and dispose of as required by local regulations.

Fix gels

1 After electrophoresis (*UNITS 10.1–10.4*), place gels in a glass or plastic container and wash in deionized water for 5 min on a platform shaker with continuous, gentle agitation.

Since silver nitrate reacts with skin proteins, wear gloves that have been thoroughly rinsed with deionized water when handling all materials. All reagents should be removed from staining trays using vacuum aspiration to avoid any contact between skin or gloves and gels, as any physical contact with the gels can create staining artifacts.

IMPORTANT NOTE: *All subsequent incubations and washes are performed with continuous gentle agitation on a platform shaker as described above.*

2 Remove water and add ~10 to 20 gel volumes fixing solution A. Fix gel by incubating for 1 hr in this solution.

3 Remove fixing solution A and add ~10 to 20 gel volumes fixing solution B. Fix gel by incubating for 2 hr to overnight in this solution.

4 Remove fixing solution B and wash with ~10 to 20 gel volumes deionized water for 5 min.

5 Remove water and incubate gel in ~5 to 10 gel volumes 0.5 M sodium acetate containing 1% glutaraldehyde for 30 min.

CAUTION: *Glutaraldehyde is an irritant. Wear gloves and prepare solution in a fume hood.*

6 Carefully remove glutaraldehyde solution and wash gel with three changes of ~10 to 20 gel volumes deionized water, each time for 10 min.

7 Remove water and incubate gel in two changes of ~5 to 10 gel volumes 0.5% (w/v) 2,7-napthalene disulfonic acid, each time for 30 min.

The solution in these steps provides homogeneous dark brown staining of proteins.

8 Remove the 2,7-napthalene disulfonic acid solution and rinse gel with four changes of ~10 to 20 gel volumes deionized water, each time for 15 min.

Stain gels

9 Remove water and stain gel with ~5 to 10 gel volumes of freshly prepared ammoniacal silver nitrate solution for 30 min.

10 After staining, wash the gel with four changes of ~10 to 20 gel volumes deionized water, each time for 4 min.

11 Remove water and develop gels in ~5 to 10 gel volumes citric acid developing solution for 5 to 10 min.

Develop gels until a slight background stain appears and bands are a homogeneous brown color; be careful not to overdevelop. As noted above, all gels that will be used for quantitative comparisons should be processed and developed using identical conditions.

12 When gels are fully developed, quickly remove citric acid solution and stop development by incubating for 30 min in ~10 to 20 gel volumes stop solution.

13 Remove stop solution and rinse gel with ~10 to 20 gel volumes deionized water for at least 30 min.

Record and store gels

14 Scan gels using a flatbed scanner (see Support Protocol).

Gels should be scanned promptly after development, i.e., within about 24 hr, because the stained spots may fade with time of storage and/or background may increase with time.

15 Store gels in resealable plastic bags at 4°C after scanning.

ALTERNATE

PROTOCOL 3

NONAMMONIACAL SILVER STAINING—The nonammoniacal silver staining procedure is similar to Basic Protocol 3 except that more stable solutions are used for staining. Using this procedure, some proteins may be detected that are not recognized by other silver staining methods.

Materials: Fixing solution A: 50% (v/v) methanol/10% (v/v) acetic acid (store up to 1 month at room temperature)

Fixing solution B: 5% (v/v) methanol/7% (v/v) acetic acid (store up to 1 month at room temperature)

10% (w/v) glutaraldehyde

5μg/ml dithiothreitol (DTT; make fresh daily)

0.1% (w/v) silver nitrate

Carbonate developing solution (see recipe)

2.3 M citric acid

0.03% (w/v) sodium carbonate

Plastic or glass container with tight-fitting lid, of size appropriate for gel

Platform shaker

Fix and stain proteins

1. Remove gel from electrophoresis apparatus and place in a plastic or glass container equipped with a lid. Add 5 to 10 gel volumes fixing solution A. Agitate for 30 min on a platform shaker.
2. Decant fixing solution A, and add 5 to 10 gel volumes fixing solution B. Agitate for 30 min.
3. Remove fixing solution B, add 50 ml of 10% glutaraldehyde, and agitate for 10 min in a fume hood.

   CAUTION: *Glutaraldehyde is an irritant. Wear gloves and work only in a fume hood*.
4. Decant glutaraldehyde solution. Add 5 to 10 gel volumes water and agitate for 15 min. Repeat eight times.

   Alternatively, wash gel overnight in 30 to 40 gel volumes water with slow agitation. The next day add fresh water and agitate for 30 min to ensure that all traces of glutaraldehyde are removed so a clear background is attained.
5. Decant water and add 5 gel volumes of 5 μg/ml DTT. Agitate 30 min.
6. Pour off DTT and, without rinsing, add 5 gel volumes of 0.1% silver nitrate. Agitate 30 min.

Develop stain and process gel

7. Decant silver nitrate solution. Rinse gel once for 15 sec with water by filling the container with water and then pouring it out. Rinse gel quickly two times with a few milliliters of carbonate developing solution. Finally, add 100 ml carbonate developing solution and agitate until bands appear as desired.
8. When proper staining has been attained, add 5 ml of 2.3 M citric acid directly to the carbonate developing solution to stop the staining reaction. Agitate for 10 min, replace the solution with water, and agitate for 30 min. Repeat four more times.

   Always use a 1:20 (v/v) ratio of 2.3 M citric acid to carbonate developing solution to neutralize the solution, thus stopping the reaction. If the pH is too high, color development will continue; if the pH is too low, the color will bleach away.
9. To store gel, soak the gel in 5 to 10 gel volumes of 0.03% sodium carbonate for ~1 hr with agitation to prevent bleaching, then place in a resealable plastic bag. Store gel at 4°C.
10. To scan or photograph gel, see Support Protocol. To dry gel, see Basic Protocol 1 (step 6).

ALTERNATE

PROTOCOL 4

RAPID SILVER STAINING—Unlike the above silver staining procedures (see Basic Protocol 3 and Alternate Protocol 3) which use glutaraldehyde as a fixative, rapid silver staining employs formaldehyde to fix the gel. The procedure has a sensitivity similar to that of the previous protocols. Also, the developing solution contains thiosulfate to inhibit background staining by dissolving unspecific surface staining. This method is rapid but may not be quiteas sensitive as other methods, especially for small proteins.

Materials: 50% methanol/12% acetic acid (v/v)

95% ethanol (optional)

Formaldehyde fixing solution (see recipe)

0.02% (w/v) sodium thiosulfate

0.1% (w/v) silver nitrate

Thiosulfate developing solution (see recipe)

50% methanol

Plastic or glass container with tight-fitting lid, of size appropriate for gel

Platform shaker

Fix and stain proteins

1. To prevent protein diffusion and improve background, fix gel after electrophoresis, if desired, for a minimum of 1 hr to overnight in 50% methanol/12% acetic acid. Wash gel with 95% ethanol three times, 20 min each time, before proceeding with step 2.
2. Add 3 gel volumes formaldehyde fixing solution. Place container on a platform shaker, then agitate 15 min for a 1-mm-thick gel or 30 min for a 1.5-mm-thick gel.
3. Decant fixing solution, then wash gel by adding 5 to 10 gel volumes water and agitating for 5 min. Repeat two times.
4. Remove water and add 3 gel volumes of 0.02% sodium thiosulfate. Agitate for 1 min.
5. Decant sodium thiosulfate and add 5 to 10 gel volumes water. Agitate for 1 min. Repeat two times.
6. Decant water and add 3 gel volumes of 0.1% silver nitrate. Agitate for 10 min.

Develop stain and process gel

7 Remove silver nitrate, add 5 to 10 gel volumes water, and agitate for 1 min. Repeat two times.

8 Decant water. Add a few milliliters of thiosulfate developing solution until a brown precipitate forms (30 sec), decant, and add 3 gel volumes fresh thiosulfate developing solution.

9 Just before bands reach desired intensity, wash the gel quickly with water to remove excess thiosulfate (to avoid background staining). Stop the reaction by adding 50% methanol/12% acetic acid and agitate for 10 min.

10 Wash the gel with 5 to 10 gel volumes of 50% methanol with agitation for ~30 min to remove acetic acid. Repeat one time.

The methanol washes are important to prevent darkening of protein bands over a prolonged period.

11 Store gel in a resealable plastic bag at 4°C.

12 For a permanent record, scan or photograph gel (see Support Protocol) or dry gel (see Basic Protocol 1, step 6).

ENHANCED-BACKGROUND (TWO-STAGE) RAPID SILVER STAINING—The enhanced-background rapid silver staining method is a two-stage staining procedure that enhances the sensitivity of most proteins and decreases background staining. In this method, however, proteins are not chemically cross-linked by either glutaraldehyde or formaldehyde; hence some proteins, especially those of low molecular weight, may be selectively lost during staining. The first part of the procedure is a rapid silver staining method that is easier and quicker than both basic silver staining and nonammoniacal silver staining. The second part of the procedure, which is optional, improves sensitivity and lessens background staining of the gel.

Materials: Rapid fixative 1: 40% methanol/10% acetic acid (v/v; store 1 month at room temperature)

Rapid fixative 2: 10% methanol/5% acetic acid (v/v; store 1 month at room temperature)

1× oxidizer (see recipe)

0.17% (w/v) silver nitrate

Thiosulfate developing solution (see recipe)

5% (w/v) acetic acid

Farmers reducer (see recipe)

Plastic or glass container with tight-fitting lid, of size appropriate for gel

Platform shaker

Rapid Fixation Silver Staining

Fix proteins

1. After electrophoresis, soak gel in 5 gel volumes rapid fixative 1 in a glass or plastic container for 60 min, agitating container on a platform shaker.
2. Pour off used rapid fixative 1 and add 5 gel volumes rapid fixative 2. Agitate for 30 min.
3. Pour off used rapid fixative 2 and add another 5 gel volumes fresh rapid fixative 2. Agitate for 30 min.

Stain and develop gel

4. Pour off used rapid fixative 2 and add 100 ml of 1× oxidizer. Agitate for 10 min.
5. Pour off oxidizer, add 5 gel volumes water, and agitate for 15 min. Repeat four times.
6. Add 100 ml of 0.17% silver nitrate and agitate for 45 min.
7. Wash gel with running water for 2 min.
8. Add 1 gel volume thiosulfate developing solution. After a brown precipitate appears (~30 sec), decant solution and add 5 gel volumes fresh thiosulfate developing solution.
9. After protein bands have reached the desired intensity, stop the reaction by pouring off developing solution and replacing with 5% acetic acid.
10. Pour off acetic acid, add 5 gel volumes water, and agitate 15 min. Repeat three times.
11. To improve background and sensitivity, continue to step 12. Otherwise, store the gel at 4°C in a resealable plastic bag with 5% acetic acid. Alternatively, scan or photograph (see Support Protocol) or dry the gel according to step 6 in Basic Protocol 1.

Improving Sensitivity (Optional)

12. Remove water, add 5 gel volumes Farmers reducer, and agitate for 10 min.

    After this step the gel should be transparent and yellow, and void of any silver-stained bands.

13. Remove reducer and wash gel by adding 5 gel volumes water and agitating for 15 min. Repeat wash five times.

    The gel must be colorless before continuing; therefore, it is preferable to do the last wash overnight.

14. Repeat steps 6 to 10. Store the gel in a sealed plastic bag at 4°C; scan or photograph (see Support Protocol), or dry the gel (see Basic Protocol 1, step 6) for a permanent record.

SUPPORT

PROTOCOL

GEL IMAGING—Historically, gel images were obtained using Polaroid or 35-mm photography. These older techniques have been largely replaced by digital-imaging methods, which have become reasonably economical and facile. Flatbed scanners in particular, are economical and easy to operate. Digital images are essential for quantitative analyses of gel images; they also facilitate data archiving and transfer over the Internet, and streamline illustration of data in presentations and manuscripts. The choice of imaging method depends on the types of imaging devices available, the quality of image desired (resolution and bit depth), and the type of stain used (colorimetric or fluorescent). The critical factors to consider in acquiring digital gel images are briefly summarized here. For a more detailed discussion of digital imaging, see *UNIT 10.12*.

Resolution, Bit Depth, and File Size: Most instruments used for digitizing gel images offer multiple options for spatial resolution and bit depth. Using the highest resolution and bit depth available is not always the best option, primarily because file sizes can become very large, which not only results in consumption of large amounts of disk space but can slow performance of subsequent analysis programs. Hence, when setting image acquisition parameters, the requirements of downstream analysis software should be considered.

The required spatial resolution of the acquired image will be influenced by the purpose of the resulting images. Applications with low to moderate resolution requirements include preparation of laboratory notebook prints, and Power- Point figures. Higher resolution is generally required when software programs will be used to identify and quantitate bands and spots on 1-D and 2-D gels or for publication in journals. For these applications, sufficient detail must be captured so that the smallest and highest-resolution features are preserved and do not limit the capacity of the quantitation software. In some cases, spatial resolution may be influenced by software requirements or limitations of the imaging system used. However, as a general guide, 2-D gel images should be acquired using a spatial resolution that yields at least 15 to 20 data points (pixels) for the smallest spot, and the sharpest band on a 1-D gel should contain at least 6 to 10 pixels of data across the band. Depending upon the imaging device, resolution will be specified in either micrometers (μm) or dots per inch (dpi). For most purposes, images of minigels at 50 or 100 μm or 300 to 600 dpi (~85 μm to 42 μm) are adequate.

Signal intensity resolution is dependent upon the resolution capacity of the detection device and the bit depth, or the number of bits used to define each pixel of a digitized image, and determines the maximum quantitative resolution of signal intensity or number of grayscale units (Miller et.al. 2006) The most common options are 8-, 12-, or 16-bit resolution. The major advantage of 8-bit resolution, which provides 256 grayscale units, is small file size. This format was compatible with older imaging systems and software but provides very limited quantitative dynamic range. It is adequate for photographic-type prints and computer-generated displays, but is not optimal for quantitative analyses. Some imaging systems and software use 12-bit data with 4096 grayscale levels; however, due to the

availability of faster computers and economical data storage devices, 16-bit data with 65,536 gray scale units has become the most common, preferred format.

The most common digital imaging devices include flatbed scanners, cooled CCD cameras, CCD cameras, and laser scanners. Brief summaries of each imaging source are given below. It is important to note that, for all of these methods, all gels must be destained properly for best results; otherwise signal-to-noise will not be optimal.

Flatbed Scanners: A simple, inexpensive, effective method of acquiring digital images of Coomassie, colloidal Coomassie, or silver-stained gels is to use a flatbed scanner in transparency rather than reflective mode. Data quality will be substantially compromised if images are acquired in reflective mode. Scanning resolution is typically specified in dpi rather than micrometers. Scanners typically offer greater uniformity of illumination and provide more reliable density analyses (Miura, 2001).

Charged-Coupled Device (CCD) Cameras: CCD cameras usually offer illumination sources for both colorimetric and fluorescent images(Miura, 2003). These cameras provide high resolution and signal intensity by dissecting an image into many small picture elements, or pixels, which are stored on a CCD chip and later converted to a video output (Miller et al., 2001). Most imaging devices offer a range of 50-, 100-, or 250- μm resolution. Inexpensive units do not use cooling of the CCD chip, often have manual adjustments for focus and image size, and may have simple software that does not adjust for non-uniform light source intensity and camera lens distortion. Such cameras are useful for archiving visual images but will generally have limited utility for quantitative comparisons. Cooled CCD cameras use reduced temperatures (e.g., 25–30°C below ambient temperature) to reduce background noise, thereby allowing longer exposures with associated increased sensitivity for fluorescent and chemiluminescent images. These cameras often have fixed focal distances and image sizes. Objects that are larger than the acquisition size of the camera are imaged sequentially and then combined into a composite image using software. Software is also used to correct for light-intensity differences. Another feature of these cameras is that they usually acquire images at the maximum possible resolution, and, when reduced image resolution is selected, data points are averaged. Hence, a cooled CCD camera with 50- μm resolution will average 4 data points on the 100- μm setting, which will improve signal-to-noise and reduce file size by four-fold. Therefore, a lower resolution setting can be advantageous for some imaging applications with these devices, provided that spatial resolution is still adequate for analysis software (see above). Cooled CCD cameras with appropriate excitation and emission filters will typically produce the greatest-sensitivity images when using fluorescent dyes such as SYPRO Ruby (see Basic Protocol 2).

Laser Scanners: An alternative to using a CCD camera for fluorescence imaging is to use a laser scanner. Laser-scanner-based systems pass an illumination beam over each point in a sample in a 2-dimensional fashion to excite the specific fluorophore (Patton, 2000). When imaging these gels, sensitivity is determined by the signal-to-noise ratio, and sensitivity can only be increased by increasing the sensitivity of the photomultiplier tube, provided the background is low (Yan, 2000). Therefore, it is essential to adequately stain the sample to

achieve maximum sensitivity, as well as to properly wash the gel to minimize background fluorescence.

Current laser-based scanners have evolved to have greater functional capabilities and use multiple lasers and emission filters to accommodate the wide variety of flourophores available (Miller, 2006). Additionally, laser scanners can now also image common visible stains such as Coomassie and silver through image digitization. This imaging principal uses a fluorescent plate as an epi-illumination background (Miura, 2003). Excitation light passes through the gel and excites the fluorescent plate. The light emitted from the plate passes through the gel again and is collected and converted to an electronic signal. For Coomassie and silver stained gels the ideal excitation source is a 532nm laser.

REAGENTS AND SOLUTIONS—*Use Milli-Q-purified water or equivalent for the preparation of all buffers. For common stock solutions, see* APPENDIX 2E; *for suppliers, see* SUPPLIERS APPENDIX.

Acid-based Coomassie blue G-250 staining solution: Prepare 0.2% (w/v) Coomassie brilliant blue G-250 (Bio-Rad) in water. Add an equal volume of 2 N H2SO4. Mix well and let stand for 3 hr minimum. Filter through Whatman no. 1 filter paper. Measure recovered volume and add 1/9 vol of 10 N KOH. Finally, add 100% (w/v) trichloroacetic acid (TCA) to a final concentration of 12% (w/v). The final concentration of Coomassie blue is ~0.08% (w/v). Store up to several months but maintain solution below pH 1.0.

Ammoniacal silver nitrate solution

Stock solution I: Dissolve 2 g of silver nitrate in 10 ml of water (prepare fresh).

Stock solution II: Mix 53 ml of deionized water, 2.8 ml of 30% ammonium hydroxide, and 0.265 ml of 50% sodium hydroxide (v/v), and stir vigorously. Prepare fresh.

CAUTION: *Wear protective glasses and take other appropriate precautions for strongly alkaline solutions (also see* APPENDIX 2A*) when handling stock solution II.*

Working solution: Slowly pipet stock solution I into solution II while the latter solution is being vigorously stirred. A transient brown precipitate will form briefly; after it clears, add deionized water to bring the total volume 250 ml.

Carbonate developing solution: 0.5 ml 37% (v/v) formaldehyde

30 g sodium carbonate (3%, w/v)

H2O to 1 liter

Make fresh each time

Citric acid developing solution: 0.05 g citric acid monohydrate (0.01% w/v final)

0.5 ml formaldehyde (0.1% w/v final)

H2O to 500 ml

Make fresh each time.

Coomassie blue R-250 staining solution: *Concentrated stock:* Mix 24 g Coomassie brilliant blue R-250 (Bio-Rad) and 600 ml methanol. Add 120 ml acetic acid. Stir for 2 hr minimum, and preferably overnight. Store up to several months at room temperature.

Staining solution: Mix 1liter methanol and 60 ml Coomassie blue R-250 concentrated stock. Add 800 ml H2O, then 200 ml concentrated acetic acid. If precipitation occurs, filter the solution through Whatman no. 1 filter paper prior to use. Store up to several months at room temperature.

The staining solution has approximate final concentrations of 50% (v/v) methanol, 0.1% (w/v) Coomassie brilliant blue R-250, and 10% (v/v) acetic acid.

Farmers reducer: 5 g potassium ferricyanide

8 g sodium thiosulfate

H2O to 500 ml

Make fresh every time

Formaldehyde fixing solution: 400 ml methanol

0.5 ml 37% (v/v) formaldehyde

H2O to 1 liter

Store ≤1 month at room temperature

Oxidizer, 10× and 1×: *10× stock*:

5 g potassium dichromate

1 ml 70% (w/v) nitric acid

H2O to 500 ml

Store up to several months at 4°C

1× working solution:

Dilute 10× oxidizer 1/10 with H2O

Make fresh each time

Polyacrylamide gel (for use in Basic Protocol 3): The basic silver staining protocol (see Basic Protocol 3) involves casting the polyacrylamide gels using piperazine-diacrylyl (PDA)

as the cross-linker to provide a gel that is more stable to the alkaline pH of the ammoniacal silver nitrate solution. These gels are also cast containing 5 mM sodium thiosulfate to reduce gel background. See *UNITS 10.1–10.4* for casting of PAGE gels; see http://world-2dpage.expasy.org for these modifications.

Thiosulfate developing solution: 15 g sodium carbonate

0.5 ml 37% (w/v) formaldehyde

0.2 ml 1% (w/v) sodium thiosulfate

H2O to 500 ml

Make fresh each time

COMMENTARY

Background Information

Coomassie blue and silver staining are the two most common approaches used for protein detection in gels with fixation. Silver staining relies on binding of silver to amino acid side chains, primarily sulfhydryl and carboxyl groups, and Coomassie blue binds nonspecifically to proteins. Silver staining is typically about 10 to 100 times more sensitive than Coomassie blue staining but it is more complex and time-consuming. There are many variations of silver stain methods both in the scientific literature and in the form of commercial kits (see Table 10.5.3) and their detection sensitivity can vary widely. Additionally, fluorescent stains for gels such as the SYPRO series first introduced in 1999 are available only as preformulated solutions (Patton, 2000). The most sensitive stain, SYPRO Ruby (see Basic Protocol 2, and Table 10.5.4), can be as sensitive as some silver stain methods when high-performance laser scanners or cooled CCD cameras are used to acquire the images. This staining method is much simpler than silver staining and uses less harsh conditions, which can be an advantage when gel bands or spots will be used to identify proteins using mass spectrometry methods. Another advantage of the SYPRO fluorescence stains is that they typically have about a three order-of-magnitude dynamic range of detection, which is much wider than the dynamic ranges for the colorimetric stains. Interestingly, it was recently suggested that several Coomassie blue stain formulations can provide increased protein detection sensitivity of < 1ng when used as infrared dyes (Butt et.al. 2013). This level of sensitivity slightly exceeds that of SYPRO Ruby and would be highly desirable when considering costs of reagents; however, this method requires sensitive infrared-capable imaging devices that may not be available to all laboratories. The Basic and Alternate Protocols described for Coomassie blue staining differ most significantly in time required. The rapid Coomassie blue G-250 staining procedure (Alternate Protocol 1) uses isopropanol for fixing instead of methanol, and the colloidal stain produces a low background, which allows for faster protein staining (bands appear within 15 min); however, it is not as sensitive as Basic Protocol 1 involving Coomassie blue R-250 (Weber et al., 1972). The acid-based Coomassie blue staining procedure (Blakesley and Boezi, 1977) also uses colloidal Coomassie blue G-250, but it does not involve a separate fixation step (Alternate Protocol

2). Acid-based staining achieves a sensitivity similar to that of Coomassie R-250, although for maximum sensitivity gels must be stained 5 hr to overnight.

Basic Protocol 2 describes an optimized protocol for SYPRO Ruby staining of 1-D and 2-D gels (IEF gels can also be stained with minor modifications) that largely follows the supplier's recommendations. However, several versions of the staining protocol exist and the impact of these variations may be at least partially dependent upon the types of gels used.

Basic Protocol 3 for silver staining (http://world-2dpage.expasy.org) is a more sensitive protein detection method compared to the nonammoniacal and rapid two-stage alternate silver staining protocols; however, the procedure uses unstable ingredients and tends to require more expensive reagents. Nonammoniacal silver staining (Alternate Protocol 3) is based on a method described by Morrissey (1981). The two-stage silver staining procedure (Alternate Protocol 5) is a modification of the method described by Gorg (1985) and consists of two parts: the first stage, involving rapid silver staining, is followed by an optional series of steps to reduce background. The rapid silver staining procedure (Alternate Protocol 4; Blum et al., 1987) is a quick and easy way to detect proteins within 50 min and, like the alternate two-stage silver staining method, uses thiosulfate to reduce background impurities.

Critical Parameters and Troubleshooting

All solutions used in preparing the polyacrylamide gels as well as the staining solutions must contain high-purity reagents. In addition, gel solutions should be filtered to avoid artifacts in the gel. This is especially important in silver staining, which is based on a photochemical process where impurities can increase background and cause artefactual staining. Powder-free gloves should be used at all times, and care should be taken to avoid any physical contact with the gels, including use of vacuum aspiration, rather than pouring off reagents.

The most common environmental contaminating proteins are keratins from skin and hair, which show up as multiple bands with molecular weights between 55 to 65 kDa, especially when high-sensitivity stains are used. If such bands appear in blank lanes where no protein was loaded, most likely the stacking gel, solubilizing buffer, and/or upper electrode buffer were contaminated. The problem can be eliminated by avoiding contact of skin and hair with gel and buffer solutions and protecting solutions and gel apparatus from airborne contamination.

There can be great variations in protein and background staining with different silver staining methods or even with seemingly minor modifications of a single protocol. In addition, these protocols are very sensitive to exact reaction conditions for critical steps, including reaction times and temperatures. In general, the most critical steps are the wash step(s) to remove excess silver reagent, the development time, effectiveness of the step that stops development, and whether or not sodium thiosulfate is added to the gel during casting (see Basic Protocol 3). Addition of sodium thiosulfate to the gel typically improves the background after staining, but increases development times.

The original SYPRO Ruby staining protocol, introduced in 1999, had a number of alternative fixative recommendations and a relatively wide range of staining times; the manufacturer had also recommended several different destaining solutions that can yield quite different results. SYPRO Ruby has since been re-formulated to be more tolerant of fixative solutions and maximize binding of the fluorescent dye to available sites on proteins (Berggren, 2002). The critical impact of fixation, staining, and washes has been evaluated to determine optimal conditions to maximize sensitivity and minimize cost of the stain (Malone, 2001 and Ahnert, 2004). The method described here (see Basic Protocol 2) is adapted from the current manufacturer's protocol and is based on reported comparisons of different methods of fixation, staining, and destaining of minigels. These results suggest that the fixative is not particularly critical, but a two-step (2 × 30 min) fixation with astronger fixative such as 40% methanol/ 10% acetic acid is advantageous, especially if the gel will be archived for potential future uses such as protein identifications by mass spectrometry. At least with some gel systems, staining for 3 hr is inadequate for maximal staining, and overnight staining yields much stronger signals. The destaining conditions also appear to be quite critical for achieving maximum sensitivity. We had previously observed that if the manufacturer-recommended destain solution of 10% methanol or ethanol is used, the background fluorescence decreases rapidly and a maximum signal-to-noise ratio is obtained within the recommended 30 min. But incubation of the gel in these solutions for longer than 30 min can markedly decrease band intensities, with complete loss of some bands after overnight incubation. When water or 2% acetic acid is used as the destain solution, the background continues to decrease for a number of hours and optimal signal-to-noise ratio is obtained after overnight destaining. While this method is slower than a dilute alcohol solution, over-destaining of bands does not readily occur.

Time Considerations

In general, Coomassie blue and SYPRO Ruby staining tends to be easier and faster than silver staining. Basic Coomassie staining of standard-sized gels (11 × 16 cm) that are 1.5 mm thick require at least 2 to 3 hr of staining followed by destaining for at least several hours to overnight. Minigels require less time, ~30 min for staining and about the same time for destaining. The rapid colloidal Coomassie blue staining procedures (Alternate Protocol 1) allow visualization of protein bands within 5 to 15 min. SYPRO Ruby staining requires an hour of fixation followed by overnight incubation with the stain, and minimal (30 min) destain. Silver staining, on the other hand, is very time consuming because the gels must be treated with many different solutions. Also, gels require successive washes with water to remove glutaraldehyde for good background in the silver staining and nonammoniacal silver staining methods (Basic Protocol 3 and Alternate Protocol 3), and to remove Farmers reducer in the second part of the two-stage silver staining procedure (Alternate Protocol 5). Rapid silver staining (Alternate Protocol 4), however, is the easiest and the most time-efficient of all the silver staining methods as it takes only ~50 min.

In general, using a pre-manufactured gel stain will significantly cut down on solution preparation time, and will provide highly consistent results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 10.5.1

Overview of Common Gel Staining Methods

Type of Stain	Features
Coomassie stains	
Coomassie Brilliant blue (CBB)	Rapid, simple one-step stain and a simpledestain; good protein-to-protein consistency; range of ~2 orders of magnitude linear quantitation
Colloidal[a]	Rapid one-solution preparation; typically 5- to 10-fold more sensitive than conventional CBB; destain optional; minimal background
Environment-friendly	Nonhazardous; no alcohol/acid fixatives or destains; easy disposal of reagents; sensitivity and/or protein-to-protein stain consistency may be somewhat reduced
Rapid stains	Bands usually develop in less than 1h, sensitivity is often somewhat reduced
Silver stains	
Conventional	~100-fold more sensitive than CBB for most proteins but much greater protein-to-protein variation in staining; kits contain multiple components and also require some common reagents that are not supplied; linear quantitation range is ~1 order of magnitude; band intensities and background can vary substantially with small differences in reaction times and temperatures
Mass-spectrometry compatible	Milder chemical conditions used to minimize protein modifications; often less sensitive than conventional silver stains; reduced interference with trypsin digestion; and a greater percentage of tryptic peptides are unmodifiedtherefore can be identified by mass spectroscopy
Rapid stains	Reduced number of steps and reaction times; total staining times ~1 to 2 hr
Fluorescent dyes	
SYPRO Ruby[a]	Rapid, single-solution stain; low protein-protein variability; mass-spectrometry compatible; linear range over 3 orders of magnitude; staining sensitivity is highly dependent upon method used to acquire fluorescent image (see Basic Protocol 2 and Support Protocol); can rival silver staining sensitivity (1 to 2 ng/band); compatible with 1- and 2-D SDS-PAGE and IEF gels; available only as preformulated stain
SYPRO Orange[a]	Good sensitivity (4 to 8 ng/band); applicable to 1-D SDS-PAGE
SYPRO Red[a]	Good sensitivity (4 to 8 ng/band); applicable to 1-D SDS-PAGE
SYPRO Tangerine[a]	Good sensitivity (4 to 8 ng/band); applicable to 1-D SDS-PAGE, blotting

[a]Colloidal Coomassie and SYPRO stains typically consist of a single stock staining reagent provided by the manufacturer. Any additional fixative or destaining steps may require common laboratory reagents. Silver stain kits generally consist of multiple components which may also require supplemental reagents.

Table 10.5.2

Representative Common Coomassie Stains

Category	Representative Vendors	Stain	Limit of Detection	Time	MS compatible
Conventional	BioRad, Sigma, G Biosciences, MP Biomedicals	Brilliant Blue R-250	8–10 ng	1– 3 hr	yes
	Sigma, G Bio-sciences, MP Bio-medicals	Brilliant Blue G-250	8–10 ng	1–3 hr	yes
	Thermo Fisher, Sigma, G Bio-sciences	Colloidal Blue Stain	<10 ng	4h	yes
Environment-Friendly	BioRad	QC Colloidal Coomassie	3 ng	1–20 hr	yes
		Bio-Safe Coomassie G-250	8–28 ng	60 min – 2.5 hr	yes
	Thermo Fisher	Simply Blue™ Safe Stain	>7 ng	12 min[a]/3hr	yes
		GelCode Blue Safe Protein Stain	9 ng	2 hr 15 min	yes
	Sigma	EZBlue™ Gel Staining Reagent	5 ng	2 hr 15 min	yes
	G Biosciences	LabSafe GEL Blue™	4–8 ng	5–10 min/up to 1 hr	yes
	Lonza	ProSieve™ EX Safe Stain	<10 ng	15 min[a]/90 min	yes
	Thermo Fisher	GelCode Blue Stain Reagent	8 ng	10 min[a]/2 hr 15 min	yes
	Amresco	Blue Bandit™ Protein Stain	<20 ng	1hr 45 min	yes
Rapid	Thermo Fisher	GelCode Blue Stain Reagent	8 ng	10 min[a]/2 hr 15 min	yes
		Imperial Protein Stain	3 ng	10 min[a]/2 hr 15 min	yes
		PageBlue Protein Stain	5 ng	30 min[a]/90 min	yes
	G Biosciences	RAPIDstain™	4–8 ng	5–10 min/up to 1 hr	yes
	Diversified Biotech	Rapid Coomassie Stain	8 ng	50 min	yes

[a]Shortened staining times (>30min) require microwaving the gel while submerged in the stain. This method reduces staining times, but may also reduce sensitivity and care should be taken to not overheat the staining solution.

Table 10.5.3

Representative Common Silver Stain Kits

Representative Vendors	Stain	Limit of Detection	Time	MS Compatible
BioRad	Dodeca Silver Stain Kit	0.25–0.5 ng	3 hr	yes
	Silver Stain Plus Kit	0.6–1.2 ng	1.5 hr	yes
	Silver Stain	0.6–1.2 ng	2 hr	
Thermo Fisher	Pierce Silver Stain for Mass Spec	0.25 ng	1 hr 13 min	yes
	Pierce Silver Stain Kit	0.25 ng	2 hr 25 min	yes
	Pierce Color Silver Stain Kit	0.1 ng	4 hr 5 min	yes
	SilverXpress® Silver Staining Kit	0.86 ng	2 hr	yes
Sigma	ProteoSilver™ Plus Silver Stain Kit	0.1 ng	1 hr 45 min	yes
	ProteoSilver™ Silver Stain Kit	0.1 ng	1 hr 45 min	yes
GE Healthcare	PlusOne Silver Staining Kit, Protein	0.2–0.6 ng	2 hr	yes[a]
G Biosciences	FASTsilver™	1 ng	1 hr	yes
	FOCUS™ FASTsilver™	1 ng	>90 min	yes
Amresco	Silver Bullit™ Silver Stain Kit	>1 ng	4 h 20 min	yes

[a]This kit is compatible with mass spectrometry only when glutaraldehyde is omitted from the staining protocol.

Table 10.5.4

Representative Common Fluorescent Stains

Representative Vendors	Stain	Limit of Detection	Time	MS Compatible
BioRad	Flamingo Fluorescent Gel Stain	0.25–0.5 ng	5 hr	yes
	Oriole Fluorescent Gel Stain	0.5–1 ng	1.5 hr	
Thermo Fisher	Coomassie™ Fluor Orange	8 ng	1 hr	yes
	Krypton Fluorescent Protein Stain	0.25 ng	2 hr 40 min	
Thermo Fisher; Sigma; BioRad; Lonza	SYPRO® Ruby	0.25 ng	90 min[a]/18 hr	yes
Thermo Fisher; Sigma	SYPRO® Orange	4–8 ng	~1 hr	yes
Thermo Fisher; Lonza	SYPRO® Red	4–8 ng	~1 hr	yes
	SYPRO® Tangerine	4 ng	~1 hr	yes

[a]The 90 min rapid protocol requires microwaving the gel and staining solution for 3 × 30 sec, or alternatively incubating the gel at 80°C for 30 min followed by subsequent destaining. This protocol will result in decreased maximum fluorescence signal compared to the overnight protocol, however it will also result in lower background, thus enabling ~0.25ng sensitivities on 1-D gels.