DETECTION OF PROTEINS

The protocols in this section require that detectable proteins have been previously separated using either one-dimensional (*UNIT 10.2*) or two-dimensional (*UNITS 10.3, 10.4*) electrophoresis. *UNIT 10.6* describes detection by Coomassie Blue or silver staining of protein-containing bands or spots in a gel. Alternatively, proteins in a gel may be electrophoretically transferred to a blot transfer membrane (*UNIT 10.8*). The proteins transferred to the membrane may be detected by staining with India ink, colloidal gold, or colloidal iron (*UNIT 10.7*), or by Western blotting (*UNIT 10.8*).

Detection of Proteins in Gels

COOMASSIE BLUE STAINING

The detection of protein bands in the gel by Coomassie Blue staining depends on nonspecific binding of a dye, Coomassie Brilliant Blue R, to proteins. The detection limit is 0.3 to 1 μg/protein band.

Materials

Fixing solution
Coomassie Blue solution
Destaining solution
7% aqueous acetic acid (vol/vol)

Plastic boxes
Cellophane membrane backing (Bio-Rad)

NOTE: *Deionized, distilled water should be used throughout this protocol.*

1. Place the polyacrylamide gel(s) in a plastic box and cover the gel with fixing solution for 2 hr. Agitate slowly on an orbital shaker.

   If agitation is too rapid, the gel may break apart. Use fixing solution only once.

2. Pour out fixing solution. Cover the gel with Coomassie Blue solution for 4 hr. Agitate slowly.

   Use staining solution only once.

3. Pour out staining solution. Rinse gel briefly with fixing solution. Cover with destaining solution for 2 hr. Pour out destaining solution. Agitate slowly.

4. Continue destaining until blue bands and a clear background are obtained. Store gels in 7% acetic acid or water.

   Do not store gels in fixing solution; the protein bands will eventually disappear.

5. To maintain a permanent gel record, the gel may be dried. Place the gel between two cellophane membrane backing sheets that are slightly larger than the gel. Place this sandwich between two sheets of Whatman 3MM filter paper and dry in a conventional gel dryer at ~80°C for 60 min.

SILVER STAINING

The detection of protein bands in a polyacrylamide gel by silver staining depends on binding of silver to various chemical groups (e.g., sulfhydryl and carboxyl moieties) in proteins. The detection limit is ~2 to 5 ng/protein band.

Additional Materials

10% glutaraldehyde (freshly prepared from 50% stock; Kodak)

Silver nitrate solution
Developing solution
Kodak Rapid Fix, Solution A

Agitate gel slowly during steps 1 to 4.

1. Place the polyacrylamide gel in a plastic box on an orbital shaker and add fixing solution for 30 min.
2. Fix in destaining solution for at least 60 min.

   No destaining is taking place in this step; fixation continues using the same solution as was used for destaining in the Coomassie Blue staining protocol (p. 10.6.1).
3. Fix in 10% glutaraldehyde for 30 min.
4. Wash gel 4 times with water, at least 30 min each wash.
5. Stain the gel with silver nitrate solution for 15 min with vigorous shaking.

   CAUTION: *Dispose of the ammoniacal silver solution immediately, since it becomes explosive upon drying.*
6. Transfer the gel to another plastic box and wash 5 times with water (exactly 1 min for each wash).
7. Prepare developer by diluting 25 ml developing solution with 500 ml water. Transfer the gel to another plastic box, add developer, and shake vigorously until the bands appear as intense as desired. If the developer turns brown change to fresh developer.
8. Transfer to Kodak Rapid Fix for 5 min.

   If necessary, swab gel surface with soaked cotton to remove residual silver deposits.
9. Wash gel exhaustively in water to remove Rapid Fix.
10. To maintain a permanent gel record, the gel may be dried. Place the gel between 2 cellophane membrane backing sheets which are slightly larger than the gel. Place this sandwich between 2 sheets of Whatman 3MM filter paper and dry in a conventional gel dryer at ~80°C for 60 min.

REAGENTS AND SOLUTIONS

NOTE: *Deionized, distilled water should be used to prepare all solutions.*

Coomassie Blue solution

50% methanol
0.05% Coomassie Brilliant Blue R
10% acetic acid
40% H_2O

Dissolve the Coomassie Brilliant Blue R in methanol before adding acetic acid and water.

Destaining solution

5% methanol
7% acetic acid
88% H_2O

Developing solution

0.5 g sodium citrate
0.5 ml 37% formaldehyde solution (Kodak)
H_2O to 100 ml

Fixing solution

50% methanol
10% acetic acid
40% H_2O

Silver nitrate solution

Add 3.5 ml concentrated NH_4OH (~30%) to 42 ml of 0.36% NaOH and bring the volume to 200 ml with H_2O. Mix with a magnetic stirrer and slowly add 8 ml of 19.4% (1.6 g/8 ml) silver nitrate.

If the solution is cloudy, carefully add NH_4OH until it clears. The solution should be used within 20 min.

COMMENTARY

Background Information and Literature Review

Coomassie Brilliant Blue R binds nonspecifically to proteins. Since the dye does not bind to the polyacrylamide gel, proteins will be detected as blue bands surrounded by clear gel zones.

Silver staining relies on differential reduction of silver ions which is the basis for photographic processes. A highly sensitive photochemical silver staining technique (Switzer et al., 1979; Merril et al., 1984) permits the detection of polypeptides in gels at more than 100× lower concentrations than Coomassie Brilliant Blue (i.e., femtomole levels of protein). The protocol described here is based on a modified technique developed by Oakley et al. (1980) which is simpler and less expensive than the original procedures.

Critical Parameters

The high sensitivity of the silver staining technique renders it susceptible to impurities and staining artifacts. It is mandatory that the polyacrylamide gels and all staining solutions be prepared from high quality reagents in order to avoid staining artifacts. Especially important is the use of high quality water (glass distilled or deionized, carbon-filtered). The glassware used for gel polymerization and the plastic staining boxes should be cleaned thoroughly, and gels should be handled with vinyl, powder-free gloves. To avoid uneven staining of the gel surface, the polyacrylamide gel should be covered with a sheet of Parafilm in order to uniformly wet the gel surface during staining, and touched only very gently with gloved hands. If silver staining is performed infrequently in your laboratory, commercial silver staining kits should be used; those distributed by Bio-Rad and Pierce have been tested and found to be reliable and sensitive.

Anticipated Results

The sensitivity of the Coomassie Blue gel staining is ~0.3 to 1 μg/protein band; the sensitivity of silver staining is 2 to 5 ng/protein band. The sensitivity of both stains varies in an unpredictable manner with the protein being stained.

Time Considerations

Coomassie Blue staining requires approximately 8 to 12 hr. Silver staining requires ~5 hr. Fixation may be extended for several days before Coomassie Blue staining. Fixation may be extended for longer periods—up to several weeks—before silver staining.

Literature Cited

Switzer, R.C., Merril, C.R., and Shifrin, S. 1979. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.* 98:231-237.

Merril, C.R., Goldman, D., and Van Keuren, M.L. 1984. Gel protein stains: Silver stain. *Meth. Enzymol.* 104:441-447.

Oakley, B.R., Kirsch, D.R., and Morris, N.R. 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* 105:361-363.

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