Published in final edited form as:

Methods Mol Biol. 2012 ; 869: 617–620. doi:10.1007/978-1-61779-821-4_56.

A brief review of other notable protein detection methods on acrylamide gels

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Summary

Several methods have been described to stain proteins on acrylamide gels. These include ultra sensitive protein detection in one-dimensional and two-dimensional gel electrophoresis using a fluorescent product from the fungus *Epicoccum nigrum*, a fluorescence-based Coomassie Blue protein staining, visualization of proteins in acrylamide gels using ultraviolet illumination, fluorescence visualization of proteins in sodium dodecyl sulfate-polyacrylamide gels using environmentally benign, nonfixative, saline and increasing the sensitivity four- to six-fold for detecting trace proteins in dye or silver stained polyacrylamide gels using polyethylene glycol 6000. All these methods are reviewed briefly in this chapter.

Keywords

Fluorophore; Epicocconone; Visible Sypro; SDS PAGE; Blue Silver staining

1. Ultra sensitive protein detection in one-dimensional and two-dimensional gel electrophoresis using a fluorescent product from the fungus *Epicoccum nigrum*

Several protein detection methods have been devised for detection of proteins in protein gel electrophoresis. Gel protein staining with colloidal Coomassie Brilliant Blue (CBB) G-250, the best of the CBB family of stains, can detect proteins down to 6–8 ng while staining with the soluble CBB R-250 can only detect proteins down to about 50 ng and requires the additional step of destaining. Silver staining of proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) or 2-dimensional PAGE has helped attain higher sensitivity of detection in the low ng quantities of protein. Fluorescence-based detection techniques offer higher sensitivities, is safer than radioactive detection techniques,

allows for multiple labeling and fluorescence resonance energy transfer based applications, and greater stability, both prior to and after an assay (1).

Mackintosh *et al.* (1) have developed an ultra sensitive fluorescent stain 'Lightning Fast' that can detect proteins down to less than 100 pg in one-dimensional gels. The stain contains the fluorophore epicocconone, derived from the fungus *Epicoccum nigrum*, which interacts noncovalently with SDS and protein. Proteins stained with 'Lightning Fast' can be excited optimally by near-ultra violet light (about 395 nm) or with visible light (about 520 nm). Sources used in image analysis systems such as ultra violet A (about 365 nm) and ultra violet B (about 302 nm) transilluminators; Xenon-arc lamps; 488 nm and 457 nm Argon-ion lasers; 473 nm and 532 nm neodymium: yttrium aluminum garnet (Nd:YAG) solid-state lasers; 543 nm helium-neon lasers, and emerging violet, blue and green diode lasers can excite the stain. Maximum fluorescence emission of the dye is at approximately 610 nm. The new stain was found to be considerably more sensitive than SYPRO Ruby, SYPRO Orange, silver and Coomassie Brilliant Blue G-250. Staining can be accomplished in 3.5 h and is compatible with peptide mass fingerprinting using matrix assisted laser desorption ionization-mass spectrometry and Edman-sequencing (1).

2. A fluorescence-based Coomassie Blue protein staining

The SYPRO Ruby family of fluorescent dyes has high sensitivity, broad linear dynamic ranges and is compatible with mass spectrometry and other downstream protein identification (2,3). However, proteins stained by these fluorescent protein stains cannot be visualized by the naked eye. Specialized equipment, such as an ultra violet transillumination system, is required for protein detection. This greatly limits manual cutting of stained spots (4). Proteins stained by Coomassie Brilliant Blue (CBB), can be visualized by the naked eye with relatively low sensitivity, compared to fluorescent protocols.

Yang *et al.* (5) developed the visible SYPRO" staining protocol for detecting proteins following sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The authors sensitized protein gels with SYPRO Ruby and then stained the proteins using the Coomassie Brilliant Blue G-250 protocol (Blue Silver) (6). This procedure that combined the two protocols had similar or better linearity than staining with only SYPRO Ruby or Blue Silver, respectively. Also, this method was more sensitive than Blue Silver staining, simpler than staining with SYPRO Ruby, and compatible with mass spectrometry analysis (5).

3. Visualization of Proteins in Acrylamide Gels Using Ultraviolet Illumination

Kazmin *et al.* (7) describe a fast, one-step method for quick detection of proteins following sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The method depends on ultra violet-light-induced reaction of tryptophan in the presence of trichloroacetic acid, which results in fluorescent products emitting in the visible region of the spectrum. The authors show, using fluorescence spectra, the similarity of this reaction to the light-dependent reaction of chloroform with tryptophan (8,9). The ultra violet light

generated by standard transilluminators (used for detecting ethidium bromide-stained DNA) is enough to drive the required reaction to produce visible light in the protein bands that can be visualized and recorded (7).

Proteins in polyacrylamide gels are visualized rapidly by soaking in trichloroacetic acid or chloroform followed by ultra violet light illumination. In less than 20 min, protein bands can be rapidly identified on a gel using this method. The authors showed that this method is less sensitive than staining with Coomassie Blue, visualizing protein bands with as little as 1 µg of protein (a protein with typical tryptophan percentages) on thin acrylamide gels.

4. Fluorescence visualization of proteins in sodium dodecyl sulfate-polyacrylamide gels using environmentally benign, nonfixative, saline.

Protein staining often requires the use of toxic solvents such as methanol, ethanol, acetic acid, trichloroacetic acid, perchloric acid or phosphoric acid, glutaraldehyde, dichromate, formaldehyde, ammonium hydroxide, etc (10). Steinberg *et al.* report the use of SYPRO Tangerine stain that does not use any toxic reagent as the solvent (10). SYPRO Tangerine stock is diluted in phosphate buffered saline or simply 150 mM sodium chloride and used to stain protein gels in a one-step procedure (no need to destain). Proteins stained in this manner can be excited using ultra violet (about 300 nm) or visible light (about 490 nm). SYPRO Tangerine's fluorescence emission maximum is about 640 nm. The dye binds non-covalently to proteins, mediated by SDS and by hydrophobic amino acid residues to a lesser extent. The stain's sensitivity is about 4–10 ng per protein band, similar to that obtained with SYPRO Red and SYPRO orange. Colloidal Coomassie Blue staining and rapid silver staining procedures also achieve similar staining sensitivity. The advantages of using SYPRO Tangerine staining are (a) the stained proteins are not fixed and hence are amenable to easy elution procedures (b) stained proteins can be used in zymography assay if SDS does not inactivate the protein of interest (c) the stained gels can be used for western blotting and (d) the staining procedure is environmentally benign (10).

5. A Four- to Six-fold Increase in Sensitivity for Detecting Trace Proteins in Dye or Silver Stained Polyacrylamide Gels

Palumbo and Tecce (11) demonstrate the possibility of increasing or regulating the sensitivity of detecting trace proteins in gels stained by different procedures. The method is based on the use of concentrated polyethylene glycol 6000 solutions, which results in a very noticeable, reversible, and uniform size reduction of gels. Gel size reduction depends mainly on the polyethylene glycol 6000 concentration and on the acrylamide content of gels. The authors showed, by employing an appropriate polyethylene glycol 6000 concentration, the possibility of regulating the final size and then the sensitivity as required. Palumbo and Tecce (11) were able to increase the sensitivity of detection by a factor of 4–6 or more based on the conditions described above. Thus, it may be possible to eliminate the need for the cumbersome and frequently impossible pre-concentration of samples by reducing the volume of the gel in this manner. Coomassie Blue and silver stained gels were also treated in a similar manner with a concomitant increase in sensitivity. In addition, this method enables

gels with radio labeled materials to be autoradiographed in shorter times, without significant loss of resolution. A gel that is reduced in size by the use of polyethylene glycol 6000 can be restored to its original size with ease by just soaking it in the destaining solution.

Alternatively, it can be converted to a transparent glass-like plate by using isopropyl alcohol washing (11).

References

1. Mackintosh JA, Choi HY, Bae SH, et al. (2003) A fluorescent natural product for ultra sensitive detection of proteins in one-dimensional and two-dimensional gel electrophoresis. *Proteomics* 3, 2273–88. [PubMed: 14673778]
2. Chevalier F, Rofidal V, and Rossignol M (2007) Visible and fluorescent staining of two-dimensional gels. *Methods Mol Biol* 355, 145–156. [PubMed: 17093309]
3. Harris LR, Churchward MA, Butt RH et al. (2007) Assessing detection methods for gel-based proteomic analyses. *J Proteome Res* 6, 1418–1425. [PubMed: 17367184]
4. Ball MS, and Karuso P (2007) Mass spectral compatibility of four proteomics stains. *J Proteome Res* 6, 4313–4320. [PubMed: 17929854]
5. Yang Y, Wang J, Bu D, et al. (2011) A fluorescence-based Coomassie Blue protocol for two-dimensional gel-based proteomics. *Biotechnol Lett* 33, 119–121. [PubMed: 20882320]
6. Candiano G, Bruschi M, Musante L et al. (2004) Blue Silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis* 25, 1327–1333. [PubMed: 15174055]
7. Kazmin D, Edwards RA, Turner RJ, et al. (2002) Visualization of proteins in acrylamide gels using ultraviolet illumination. *Anal Biochem* 301, 91–96. [PubMed: 11811971]
8. Voropei AV, Chernitskii Ye. A., Konev SV, et al. (1992) Chloroform dependent photoproducts of tryptophan. *Biophysics* 37, 743–745.
9. Pinchuk SV, and Vorobei AV (1993) Spectral characteristics of mechanisms of forming “chloroform-dependent” tryptophan photoproducts. *J Appl Spectrosc* 59, 711–715.
10. Steinberg TH, Lauber WM, Berggren K, et al. (2000) Fluorescence detection of proteins in sodium dodecyl sulfate-polyacrylamide gels using environmentally benign, nonfixative, saline solution. *Electrophoresis* 21, 497–508. [PubMed: 10726749]
11. Palumbo G, and Tecce MF (1983) A four- to sixfold enhancement in sensitivity for detecting trace proteins in dye or silver stained polyacrylamide gels. *Anal Biochem* 134, 254–258. [PubMed: 6197903]