Protein Gel Staining

A wide range of techniques and methods for detecting proteins in gels are today available on the market, Coomassie Brilliant Blue (CBB) being one of the oldest protein gel stains, dating back to 1963. Different gel stains have different modes of interacting with proteins, making them visible over the gel matrix. CBB e.g., binds to protein via hydrophobic and electrostatic interactions[i]. Other stains bind to the SDS coat of proteins in SDS-PAGE-gels, and still others may utilise other, and sometimes more specified ways of interacting with proteins or subsets of proteins, e.g. glycolysalted proteins or phosphoproteins, opening up for a wide range of applications. Colorimetric protein stains such as CBB often present rapid and robust ways of visualising proteins in a gel, however they generally lack in sensitivity. The development of new, often fluorescent protein stains have led to protein amounts less than 1 ng being detectable when visualised using CCD-image systems.

Coomassie Brilliant Blue (CBB), an organic disulfonated triphenylmethane textile dye, was the first dye to be used for protein detection and is today the most abundantly employed protein staining method[1]. The CBB dye exists in two different forms; Coomassie R250, which has a reddish tint, and the dimethylated form, Coomassie G250, which has a greenish tint. In an acidic environment, CBB binds to both proteins and the gel matrix, via electrostatic and hydrophobic interactions. In the destaining process, dye will release faster from the gel matrix than from the proteins, leaving well defined blue visibly stained protein bands in the gel. The CBB staining procedure is relatively quick and very easy. Proteins can be retrieved unmodified from the gel after staining for subsequent processing, e.g. mass spectrometry (MS)[ii]. CBB-staining is a relatively blunt method, with a detection limit of 10-100 ng[ii], and since CBB is not an "end-point" staining method, results may vary from staining to staining. Furthermore, some proteins, like collagen, destain faster than the gel matrix, making CBB unsuitable for some applications[1]. Since the introduction as a protein dye in 1963, a large number of modifications to the original CBB-protocol have been published; among them a colloidal CBB-staining method that shows improved sensitivity and reliability compared to the basic protocol[ii].

Negative imidazole SDS zinc staining is another protein gel staining-method that generates results visible to the eye. Proteins bind to zinc ions via negatively charged amino acids[ii]. Imidazole reacts with unbound zinc ions, and a complex salt containing zinc, SDS and imidazole precipitates in the gel, forming an opaque white background. Since zinc has been bound by proteins in the protein bands of the gel, there is no precipitation there, leaving visible darker protein bands on a white background. In addition to proteins, zinc ions also bind to other biopolymers, like e.g. nucleic acids and polysaccharides[ii]. Negative staining is more sensitive than e.g. CBB, with lower detection amounts ranging from 1-10 ng. The negative zinc staining method is compatible with subsequent MS analysis after retrieving the proteins and treating them with a chelating agent such as EDTA to remove the bound zinc ions.

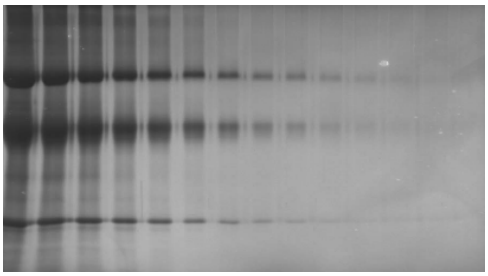
Fluorescent stains: Present protein stains frequently utilise fluorescence as a detection method. The first fluorescent protein dye to be used in SDS-PAGE –gels was SDS-binding Nile Red in 1994 [iii]. Since then a wide range of fluorescent protein stains have been developed, the largest group being the SYPRO-dyes, developed by Invitrogen/Molecular Probes (Eugene, OR, U.S.A). The SYPRO-dyes is a diverse family of protein stains, with members such as SYPRO Red, Orange, Tangerine and Ruby, all differing in mode of protein binding, fluorescence properties as well as sensitivity. Contrary to e.g. CBB, fluorescent staining is an endpoint staining and is thus highly reproducible[i]. Among the most recently developed fluorescent protein gel stains are Deep Purple Total Protein Stain (Amersham Biosciences/GE Health Care, Little Chalfont UK), which is based on a compound isolated from the fungus *Epicoccum nigrum*, that fluoresces first when interacting with protein[ii]. Fluorescent protein gel stains are generally compatible with subsequent protein analysis, e.g. MS.

Visualisation and image capture

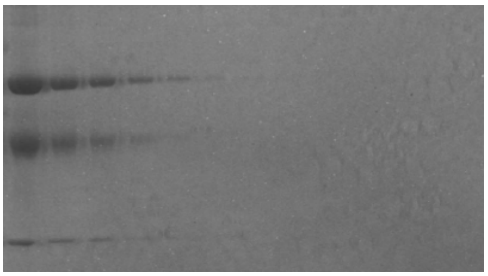
Colorimetric protein stains, such as CBB and negative zinc stain are visible to the eye once the proteins have been stained, however *analysis* of stained proteins, as bands or spots, requires for an image of the gel to be captured. Fluorescently stained protein gels frequently require UV light as excitation source, and specific filters for capturing the emitted light. Image systems, such as Wealtec's Dolphin-Doc Pro offer the convenience of housing imaging capturing devices, excitation source and dark rooms all in one system. When connected to analysis software, high resolution images can be captured and

instantly analysed using the Dolphin-1D software.

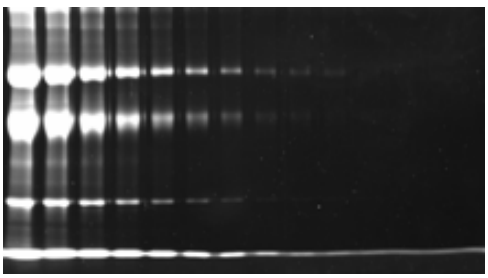
Figure 1: *Dolphin-Doc Pro Image system*



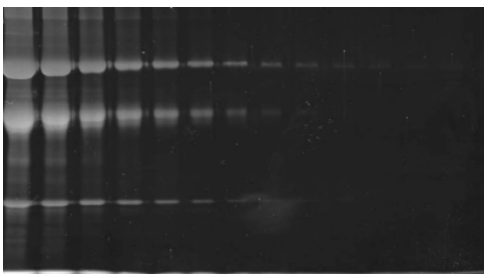
VisPro Zinc stain



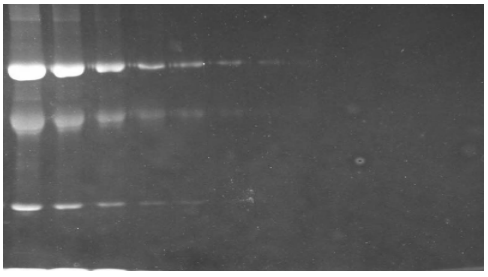
Coomassie Brilliant Blue G-250



SYPRO Ruby Basic protocol



SYPRO Ruby Rapid protocol



SYPRO Orange

Figure 2: *SDS-PAGE gels stained with different protein gel stains. 2-fold serial dilution samples of protein (BSA 66 kDa, Ovalbumine 45 kDa and Carbonic anhydrase 29 kDa, all from Sigma Aldrich, St. Louis, MO., U.S.A, were loaded onto 12 % lab-cast gels. After electrophoretic separation (pre-run 40 V and 200 V run in running buffer; 25 mM Tris-HCl pH 8.3, 192 mM glycine, 3.5 mM SDS), the gels were subjected to either CBB (BioSafe Coomassie; BioRad Laboratories, Hercules, CA, U.S.A), zinc (VisPro, Taipei, Taiwan), SYPRO Orange (Invitrogen/Molecular Probes), or SYPRO Ruby (Invitrogen/Molecular Probes)-staining, according to manufacturers instructions. When staining with SYPRO Ruby, both the rapid protocol and the basic protocol were followed. Each protein band contains, following lane 1 to 14 from left to right; lane 1: 4500 ng, lane 2: 2250 ng, lane 3: 1125 ng, lane 4: 563 ng, lane 5: 281 ng, lane 6: 141 ng, lane 7: 70 ng, lane 8: 35 ng, lane 9: 18 ng, lane 10: 9 ng, lane 11: 4 ng, lane 12: 2 ng, lane 13: 1 ng, lane 14: 0.5 ng.*
CBB and negative zinc stained gels were visualised in Dolphin-Doc Pro Image system (Wealtec Bioscience Co. Ltd., Taipei, Taiwan), using a white light converter plate (Wealtec) and UV as excitation source. SYPRO Ruby gels were visualised in Dolphin-Doc Pro (Wealtec), using UV as excitation source and a high transparent amber filter as emission filter. SYPRO Orange was visualised using a UV light and a blue light converter plate (Wealtec) to shift the excitation source closer to the excitation peak of SYPRO Orange. A high transparent amber emission filter was used. Image capture and analysis was performed using Dolphin-1D software (Wealtec).

Articles cited:

i) Westermeier R., Marouga R. (2005) *Protein Detection Methods in Proteomics Research*. Bioscience Reports 1/2:19-32

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iii) Bermudez A., Daban J. R., Garcia J. R., Mendez E. (1994) *Direct blotting, sequencing and immunodetection of proteins after five-minute staining of SDS and SDS-treated IEF gels with Nile red.* Biotechniques 4: 621-624

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