

Application Note:

Bloo Moose™ Staining Solution: Staining Sensitivity Versus Colloidal Coomassie Blue, Silver and SYPRO® Ruby

Abstract

Coomassie Brilliant Blue (CBB) staining frequently lacks the sensitivity necessary to detect low abundance proteins. While silver staining is at least ten times more sensitive than conventional CBB formulations, staining is linear only over a very narrow range and proteins may stain differentially, whereas CBB staining adheres to Beer's Law and is linear at protein concentrations over several orders of magnitude. Further, silver staining is incompatible with mass spectrometry unless modified. Fluorescent dyes such as SYPRO® Ruby would seem to bridge the sensitivity gap between CBB and silver, but require the capacity to integrate fluorescence over time to maximize sensitivity. Bloo Moose™ is a colloidal CBB stain that is at least two times as sensitive as other colloidal CBB stains. "Equilibrium staining" occurs as the dye is converted from its molecular dispersed form to a large colloid. Gels can be immediately scanned and imaged without destaining. It is estimated that SYPRO® Ruby is only two times more sensitive than Bloo Moose™. Increased sensitivity is observed in gels sequentially stained with SYPRO® Ruby and Bloo Moose™.

Introduction

Silver staining enables the detection of picogram quantities of protein in polyacrylamide gels. However, in application to proteomics, silver staining has several disadvantages including (i) staining is not cross-sectional, a requisite of quantitative staining, and may confound differential expression analyses, (ii) proteins are stained differentially or may not be stained at all, (iii) staining is linear only over a very narrow range, (iv) the aldehydes used in most methods cross-link proteins rendering them incompatible with mass spectrometry, (v) variability in washing and development times can hinder reproducibility, (vi) destaining by oxidizing the reduced silver may also oxidize proteins, and (vii)

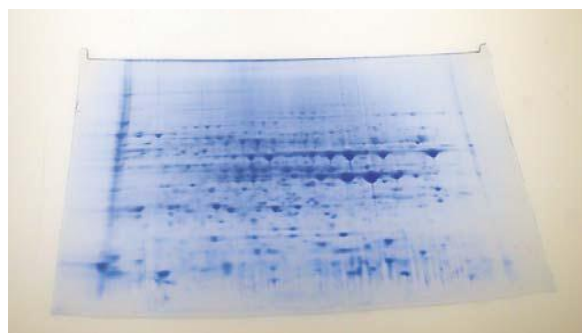


Figure 1. Staining with Bloo Moose™ results in background-free gels without the need for lengthy destaining in organic solvent.

staining is procedurally complex and produces large volumes of hazardous waste. The development of colloidal CBB stains by Neuhoff et al [1] has extended the sensitivity of Coomassie into the low nanogram range. Bloo Moose™ is a colloidal CBB stain that is at least two times as sensitive as other colloidal CBB stains and exhibits sensitivity approaching silver and SYPRO® Ruby staining

Methods and Materials

Lyophilized *Escherichia coli* strain K12, the ProteoPrep Membrane Extraction Kit, and protein standards were from Sigma Chemical Company (St. Louis, MO, USA). *Sacharomyces cerevisiae* strain s288c inoculum from ATCC (Manassas, VA) was grown to log phase in a New Brunswick Scientific BF4500 Fermenter (Edison, NJ). Bacterial and yeast cells were pelleted, then resuspended in Cellular and Organelle Membrane Solubilizing Reagent from the ProteoPrep kit and lysed by sonication. Cellular debris was removed by centrifugation and the supernatants were reduced and alkylated with 5 mM tributylphosphine and 10 mM acrylamide. Lysates were acetone precipitated to remove residual Tris and alkylating reagent and were redissolved in ProteoSolve-IEF buffer (Pressure BioSciences, inc., South Easton, MA, USA).

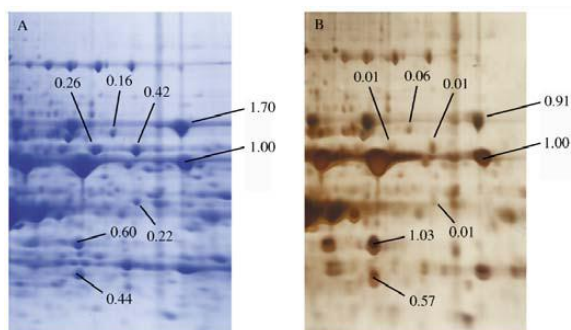


Figure 2. Differential staining of proteins on gels stained with either Bloo Moose™ or silver. The integrated density of seven protein spots is expressed as density relative to the density of the same central spot on each gel.

Protein concentration of the cell lysates was determined by Bradford assay (BioRad, Hercules, CA, USA). The concentration of the protein standards was provided by the manufacturer. For 2DGE, cell lysates were focused in broad range (pH 3-10) or narrow range (pH 4-7) immobilized pH gradients (IPGs) on the IsoelectrIQ IEF instrument. IEF proceeded at 10 kV for 100 kVh. Second dimension gels were 4-15% Criterion polyacrylamide gradient gels (Bio-Rad, Hercules, CA, USA).

Staining: Gels were stained with Bloo Moose™, GelCode Blue from Thermo-Pierce (Rockland, IL, USA), or using a modification of the Neuhoff et al. method [1] as described by Molloy et al. [2]. SYPRO® Ruby was from Life Technologies (Eugene, OR, USA). Silver staining was performed using a modification of Rabilloud et al. [3]. Gels stained with Bloo Moose™ were briefly rinsed in water and scanned without destaining. Gels stained with "environmentally-benign" colloids such as GelCode Blue were destained in water as recommended by the manufacturer. Gels stained with colloidal CBB as described by Molloy et al. were destained in 1% acetic acid until a clear background was obtained. Gels stained with SYPRO® Ruby were destained for 30 minutes in 10% methanol, 7% acetic acid. **Image Analysis:** CBB and silver stained gels were scanned on a Umax PowerLook III scanner (Umax Technologies, Dallas, TX, USA). Integrated fluorescence of SYPRO® Ruby stained gels was captured using the Alpha Innotech Fluoroimager (San Leandro, CA, USA). Signal-to-noise ratio (SNR) for each stain was calculated as described by Smejkal and Hoff [4].

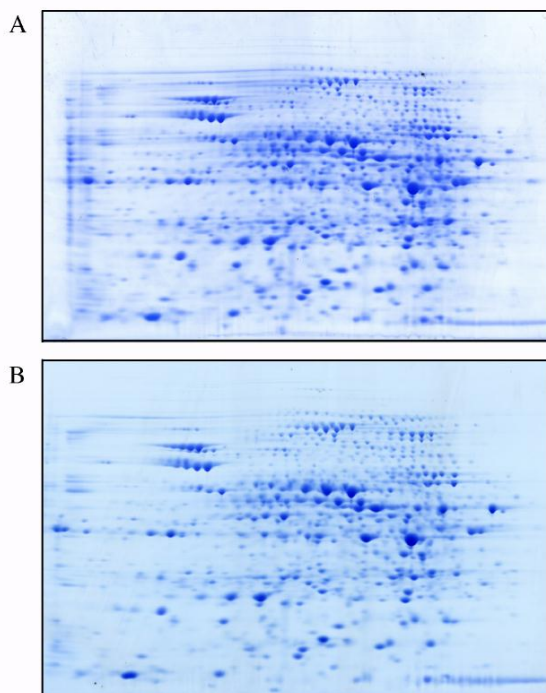


Figure 3. 2DGE of E. coli lysate. Gels were stained with either (A) Bloo Moose™ or (B) GelCode Blue. In duplicate gels, ProteomiQ Blue detected 981.5 ± 4.5 spots; GelCode Blue detected 762 ± 73 spots. IPGs were pH 4-7.

Results

Bloo Moose™ is a two-component stain that is prepared immediately before use. During the time course of staining, the dye converts to an insoluble colloid. No destaining of the gel is required. Silver staining stains some proteins differentially and this may confound differential expression analyses (Figure 2). By comparison, staining with Bloo Moose™ is linear over several orders of magnitude. Bloo Moose™ is more sensitive than other colloidal CBB stains and detected more numerous spots on 2D gels (Figure 3). Further, higher SNRs are observed without the need to destain. The SNR of Bloo Moose™ was 0.94 measured in gels taken directly from the staining solution. By comparison, gels stained by the Malloy et al. method had similar SNRs only after eight hours of destaining. The sensitivity of Bloo Moose™ is comparable to silver staining. Bloo Moose™ is more sensitive than some glutaraldehyde-free silver stains (Figure 4). Finally, the lack of need to destain the gels in organic solvent decreases the amount of hazardous waste that is generated in the laboratory.

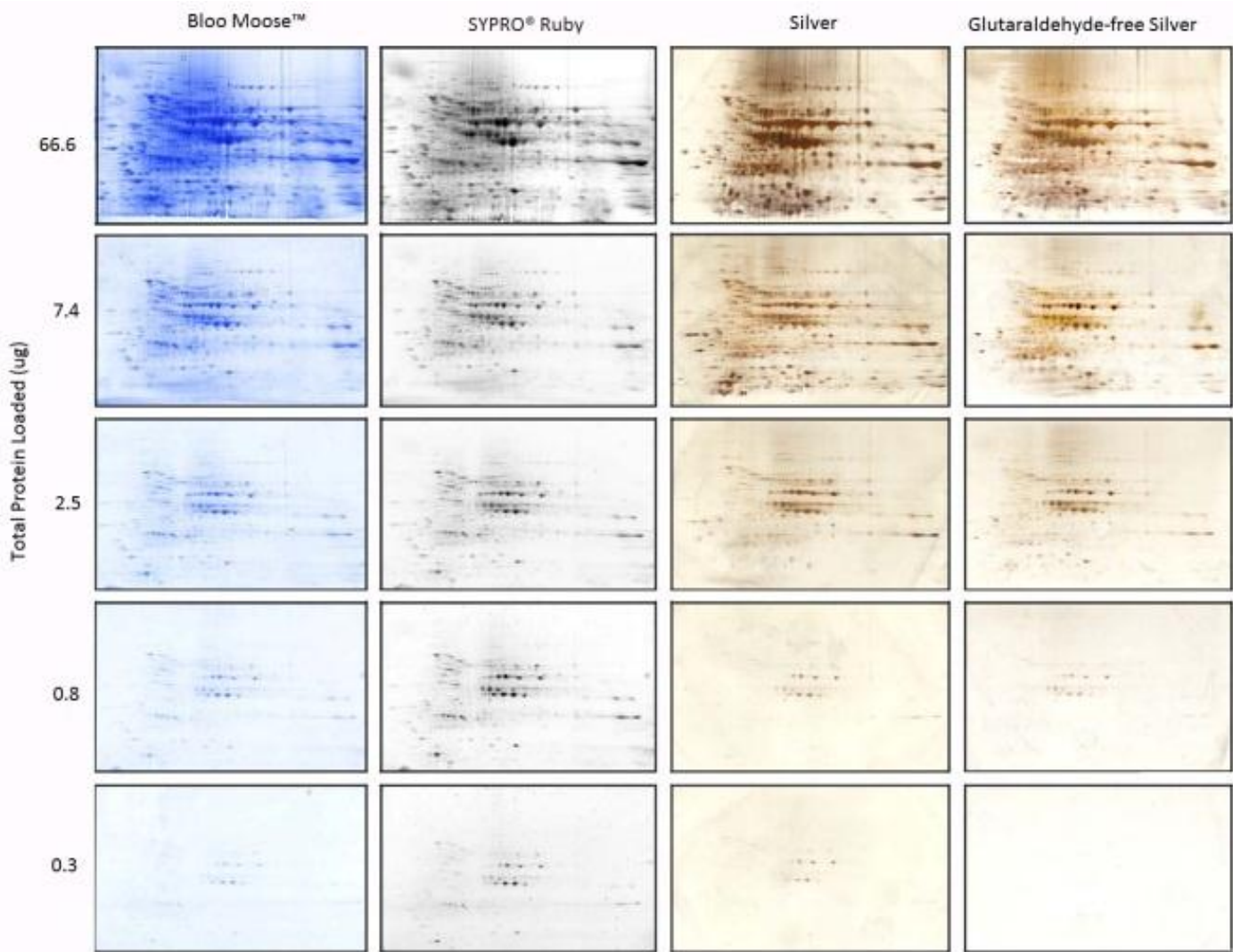


Figure 4. Serial dilutions of *S. cerevisiae* lysate at the indicated protein load were subjected to 2-dimensional gel electrophoresis and stained with Bloo Moose™, SYPRO® Ruby, or silver (below). SYPRO® Ruby fluorescence was measured at the maximum exposure time where no spots were saturated, from 5–20 seconds.

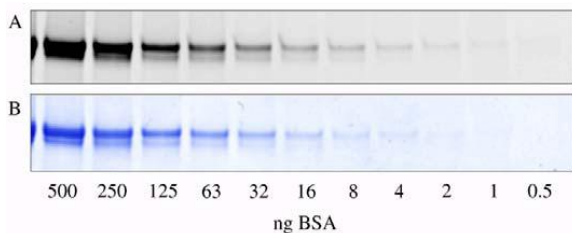


Figure 5. Serial dilutions of BSA stained with (A) SYPRO® Ruby and (B) restained with Bloo Moose™.

The sensitivity of Bloo Moose™ is comparable to SYPRO® Ruby staining as well (Figure 5). Quantitative serial dilutions demonstrated nearly identical staining pattern for bovine serum albumin down to 1 ng of protein per band.

Discussion

Bloo Moose™ is a colloidal Coomassie Brilliant Blue stain that offers the following advantages:

- It is an end-point stain, requiring no destaining steps
- It is at least two times as sensitive as conventional colloidal CBB stains
- It compares in sensitivity to conventional Silver staining and surpasses MS-friendly glutaraldehyde-free silver stain
- SYPRO® Ruby is only approximately two times more sensitive than Bloo Moose™.

References

- [1] Neuhoff et al. (1988). *Electrophoresis*, 9, 255-262.
- [2] Molloy et al. (1999). *Electrophoresis*, 20, 701-704.
- [3] Rabilloud et al. (1990). *Electrophoresis*, 11, 785-794.
- [4] Smejkal and Hoff (1994). *Electrophoresis*, 15, 922-925.

KeraFAST LLC
Richard Dean Biomedical Research Building, Suite 168
391 Technology Way
Winston-Salem, NC 27101
Telephone: 1-(800) 546-1760 or (617) 336-3228
Fax: 1-(866) 727-9826

At KeraFAST, we try our best to ensure high quality customer support.
For questions concerning your registration, order, billing, shipping information, etc., please email
CustomerService@kerafast.com

For questions concerning the performance or specifications of our products, experimental protocols and other technical issues, please email TechnicalSupport@kerafast.com
You can always reach us over the phone Monday through Friday between 8:30 AM - 5:30 PM EST:
Toll Free: 1-800-546-1760 or (617) 336-3228