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 CCB 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 that is precipitated, leaving the gel in a nearly clear solution. Gels can be immediately scanned and processed for mass spectrometry without prior 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-links 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) staining is procedurally complex and produces large volumes of hazardous waste. The development of colloidal CCB stains by Neuhoff et al [1] has extended the sensitivity of Coomassie into the low nanogram range. Bloo Moose™ is a colloidal CCB stain that is at least two times as sensitive as other colloidal CBB stains and exhibits sensitivity approaching silver and SYPRO® Ruby staining

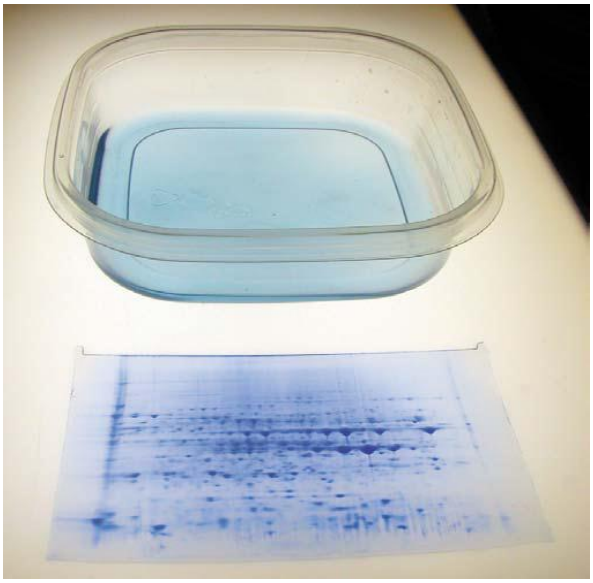

Figure 1. Conversion of the Bloo Moose ™ staining solution resulting in background-free staining of gels.

Methods and Materials

Lyophllized *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 inocculum from ATCC (Manassa, VA, USA) was grown to log phase in a New Brunswick Scientific BF4500 Fermenter (Edison, N1, USA). Bacterial and yeast cells were pelleted, then resuspended in Cellular and Organelle Membrane Solubilizing Reagent from the ProteoPrep kit and lysed by

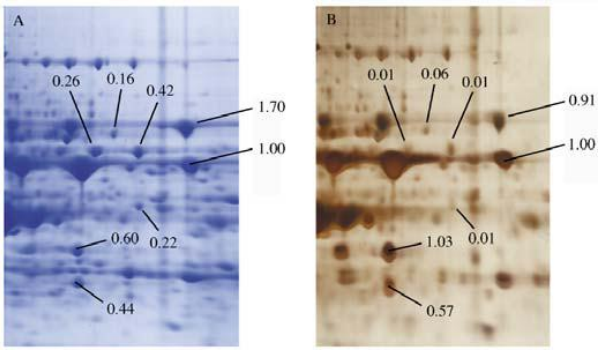

Figure 2. Differential staining or proteins on gels stained with either Bloo Moose™ or silver. The integrated density of seven protein spots is expressed as density relative to the density of the same central spot on each gel.

sonication. Cellular debris was removed by centrifugation and the supernatants were reduced and alkylated with 5 mM tributylphosphlne and 10 mM acrylamide. Lysates were acetone precipitated to remove residual Tris and alkylating reagent and were redissolved in ProteomIQ Resuspension Reagent from Proteome Systems (Woburn, MA, USA). 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. 1DGE and 2DGE 1DGE was performed in 10% ProteoGels from Sigma Chemical Company. 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 6-15% polyacrylamide gradient GelChips (Proteome Systems,Woburn, MA). Staining Gels were stained with Bloo Moose™, GelCode Blue from Pierce Chemical (Rockland, IL, USA), or using a modification of the Neuhoff et al. method [1] as described by Malloy et al. [2]. SYPRO® Ruby was from Molecular Probes (Eugene, OR, USA). Silver staining was performed using a modification of Rabilloud et al.

Gels stained with Bloo Moose™ were scanned directly from the converted staining solution without prior 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 Malloy et al. were destained in 1% acetic acid until a clear background resulted. 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®

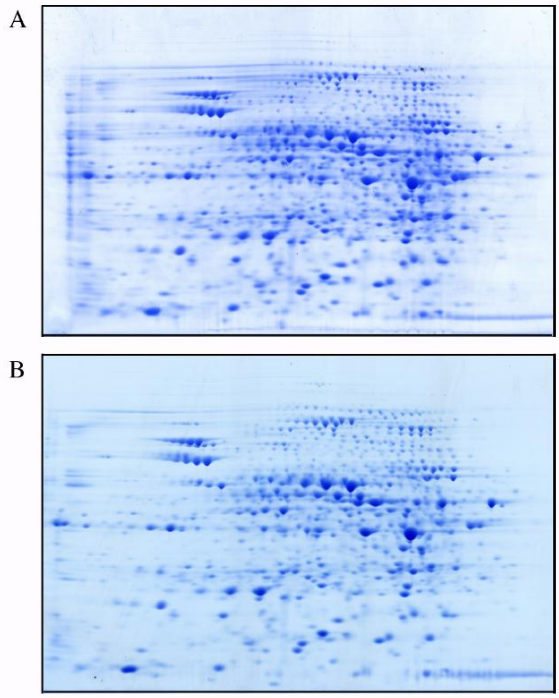

Figure 3. 2DGE of E. coli lysate. Gels were stained with either (A) Bloo Moose™ or (B) GelCode Blue. In duplicate gels, ProteomIQ Blue detected 981.5 ± 4.5 spots; GelCode Blue detected 762 ± 73 spots. IPGs were pH 4-7.

Ruby stained gels was captured using the Alpha Innotech Fluorlmager (San Leandro, CA, USA). Signal-to-noise ratio (SNR) for each stain was calculated as described by Smejkal and Hoff [4].

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 that precipitates leaving the gel in a nearly clear solution (Figure 1). No destaining is required. By spectrophotometric analyses, it was determined that 99.1% z 0.4% of dye is converted in 8 to 10 hours. 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

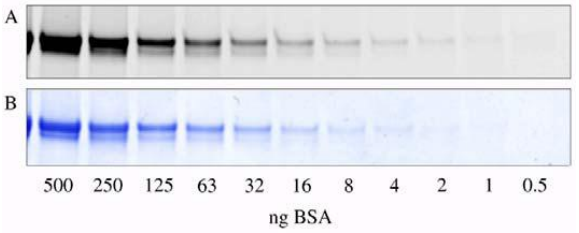

Figure 5. Serial dilutions of BSA stained with (A) SYPRO® Ruby and (B) restained with Bloo Moose™.

more sensitive than some glutaraldehyde-free silver stains (Figure 4). The sensitivity of Bloo Moose™ s 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™.

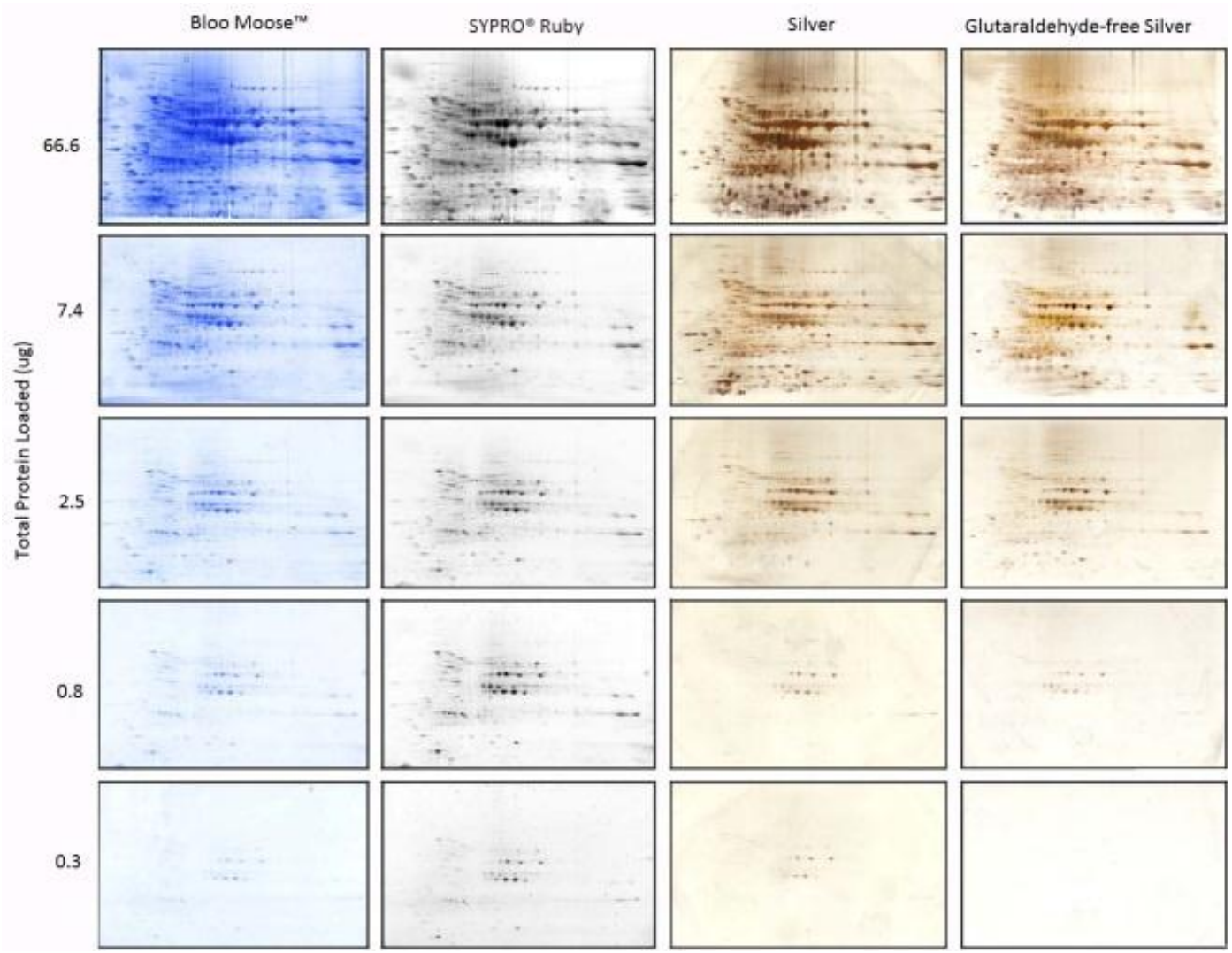

Figure 4. Serial dilutions of *S. cerevisiae* lysate at the indicated protein load were subjected to 2-dimensional gel electrophoresis and stained with Bloo Moose™, SYPRO® Ruby, or silver (below). SYPRO® Ruby fluorescence was measured at the maximum exposure time where no spots were saturated, from 5—20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

References

[1] Neuhoff et al. (1988). Electrophoresis, 9, 255-262.

[2] Malloy 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