

ProteoSilver™: High Sensitivity Silver Stain for SDS-PAGE

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Introduction

Polyacrylamide gel electrophoresis (PAGE) is a commonly used technique for analysis of proteins because of its low cost, ease of use, and high sensitivity. Following one-dimensional or two-dimensional electrophoresis of protein mixtures on a gel, the proteins are typically visualized by some form of protein staining. By far the most common protein staining solutions utilize the Coomassie® Brilliant Blue R or Coomassie Brilliant Blue G dyes. Coomassie Brilliant Blue R stains can usually detect a 50-ng protein band. Silver staining of proteins on a gel increases the sensitivity from 10-100 fold. Since the conception of silver staining of proteins in 1979,¹ many methods of silver staining have been developed. Two general classifications are 1) silver amine or alkaline method, and 2) silver nitrate or acidic method.² Detection levels of proteins using the various staining methods are determined by how quickly the background develops. The silver amine methods usually have lower background than the silver nitrate methods and, therefore, have typically been more sensitive.^{3,4} A drawback to the use of the silver diammine method is the use of glutaraldehyde for fixing and sensitization. Glutaraldehyde is not compatible with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)^{5,6,7} because it causes protein-protein cross-linkages.

The use of various types of sensitizers with the silver nitrate method has been shown to decrease the background and increase detection sensitivity.^{2,8,9} The ProteoSilver™ Kits are silver nitrate-based with a unique sensitizer solution and optimized protocols which produces better sensitivity (0.1 ng/mm²) than typically claimed using a silver amine method, but is MALDI-MS compatible. The ProteoSilver Plus Kit also contains destaining solutions for removal of silver ions from excised bands for enhanced MALDI-MS sensitivity.¹⁰

Materials and Methods

All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise noted.

Electrophoresis

Bovine serum albumin (BSA; Product Code [A 4503](#)) protein samples were prepared by serial dilutions with 1X sample buffer [62 mM Tris-HCl, pH 6.8, 10 % (v/v) glycerol, 2 % (w/v) SDS, 0.004 % (w/v) bromophenol blue, and 5 mM dithiothreitol (DTT)]. The low DTT concentration (5 mM) is typically used to reduce background staining observed with

higher concentrations of DTT. The dilutions were made from a 0.1 mg/ml BSA sample that was heated at 100 °C for 5 minutes in 1X sample buffer with 30 mM DTT. The SigmaMarker™ Wide Range (Product Code [M 4038](#)) samples were prepared by serial dilutions with 1X sample buffer. The protein samples were loaded onto 4-20 % Tris-glycine gels and electrophoresed at 170 volts using Tris-glycine-SDS running buffer (Product Code [T 7777](#)).

Silver Staining

Following electrophoresis, the gels were silver stained using either the ProteoSilver Plus Silver Staining Kit or a competitor's kit (Competitor "I"). The ProteoSilver Plus Kit and the Competitor "I" kit both utilize the silver nitrate method that is MALDI-MS compatible. The protocols designated for Tris-glycine gels were followed when using the Competitor "I" kit (Figure 1).

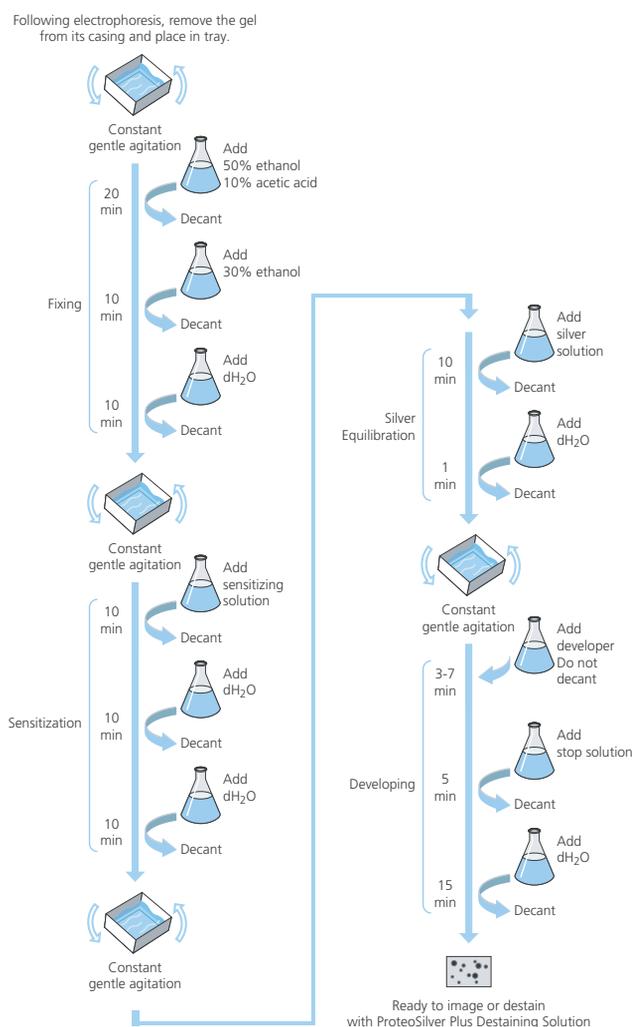


Figure 1. Overview of ProteoSilver Plus Silver Staining Kit procedure.

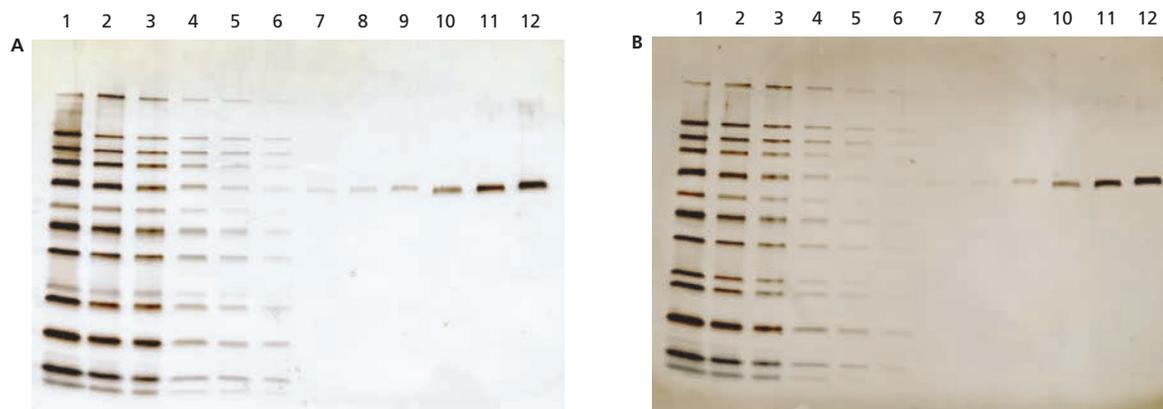


Figure 2. Comparison between the ProteoSilver Kit (A) and Competitor "I" kit (B). Both the ProteoSilver Kit and the Competitor "I" kit were used to stain a 4-20 % Tris-glycine gel. Lanes 1-6 contain decreasing loads of SigmaMarker Wide Range from 10 μ l of 100-fold dilution (Lane 1) to 2.5 μ l of 1000-fold dilution (Lane 6). Lanes 7-12: 0.35 ng, 0.5 ng, 1 ng, 2.5 ng, 5 ng, and 10 ng BSA, respectively.

Results

Comparison of Background Staining

Serial dilutions of molecular weight markers and purified BSA (0.35-10 ng) were electrophoresed on 4-20 % Tris-glycine-SDS gels and silver stained (Figure 2). The gel in Figure 2A was stained using the ProteoSilver Plus Kit from Sigma-Aldrich. The gel in Figure 2B was stained using the Competitor "I" kit. Both gels were developed until the 0.35 ng BSA band could be detected. The 0.35 ng BSA band was chosen because the Competitor "I" kit claims a detection limit of 0.3 ng. The gel stained with the Competitor "I" kit (Figure 2B) shows much higher background staining than the gel stained with ProteoSilver from Sigma-Aldrich (Figure 2A). The high background produced by the Competitor "I" kit limits the detection to approximately 0.35 ng on a 4-20 % Tris-glycine gel. The lower background of the ProteoSilver stained gel allows for lower detection limits. The detection sensitivity of ProteoSilver was explored in the following study.

Sensitivity

Lower serial dilutions of molecular weight markers and purified BSA (0.2-2 ng) were electrophoresed on 4-20 % Tris-glycine-SDS gels to determine the level of sensitivity. The gel was stained using ProteoSilver until the 0.2 ng BSA band (Lane 8) was clearly detected (Figure 3). The 0.2 ng BSA band was detected after developing the gel for 5 minutes. The area of the band is approximately 2 mm² that gives a protein density of 0.1 ng/mm². The diluted SigmaMarker proteins in Lane 6 range from 0.1-0.4 ng per band. Twelve of the thirteen protein bands were detected in Lane 6. Aprotinin (bottom band) was the only band not detectable at this dilution (approx. 0.1 ng). The upper half of the gel began to display background staining when developing beyond the 0.35 ng band.



Figure 3. ProteoSilver detection limit for BSA. Lanes 1-6 contain decreasing loads of SigmaMarker Wide Range from 5 μ l of 100-fold dilution (Lane 1) to 1 μ l of 1000-fold dilution (Lane 6). Lane 7: 2.5 μ l SigmaMarker Wide Range of 100-fold dilution. Lanes 8-12: 0.2 ng, 0.35 ng, 0.5 ng, 1 ng, and 2 ng BSA, respectively.

Discussion and Conclusion

Detection limits by silver staining are determined by how quickly the protein bands develop in relationship to the background (e.g., signal-to-noise). It should be possible to improve detection sensitivities by increasing the rate of band development, decreasing the rate of background development or both. A number of sensitizing agents have been used in the past 23 years.² Three general methods by which these sensitizers improve staining sensitivity have been proposed: 1) by binding specifically to the proteins to provide extra binding sites for silver [e.g., Coomassie Brilliant Blue], 2) by preventing background image development [e.g., dichromate] or 3) by binding to the protein forming either metallic silver or silver sulfide.⁵ The metallic silver or silver sulfide will create a latent image during the silver step so that reduction during the developing step will be faster at the protein thus increasing sensitivity. The ProteoSilver Sensitizer Solution fits into the third category.

The ProteoSilver ([PROT-SIL1](#)) and ProteoSilver Plus ([PROT-SIL2](#)) Silver Staining Kits are the most sensitive, commercially available, silver staining kits tested. ProteoSilver produces a lower background than the competition which allows for detection as low as 0.2 ng BSA (0.1 ng/mm²) on a 12 well, 4-20 % Tris-glycine gel. Neutral pH Tris-acetate gels with Tris-tricine-SDS running buffer (Product Code [I 2821](#)) have a slightly lower background with the ProteoSilver Kit allowing for detection down to 0.1 ng BSA on a 12-well gel (data not shown). The ProteoSilver and Competitor "I" kits both require approximately the same amount of time to complete the staining process (less than 2 hr).

The detection level for peptide mixtures in the range of 1-10 fmoles has been claimed using MALDI-TOF.¹¹ If a 20 % yield of peptides from a gel slice is obtained, it should be possible to analyze peptide mixtures from 5-50 fmoles of protein loaded on a gel. For BSA, 5-50 fmoles would be 0.3-3 ng protein. As previously shown, 0.2 ng BSA can be detected. Therefore, ProteoSilver Plus is able to detect the minimum amount of protein required for MALDI-MS analysis.

References

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ORDERING INFORMATION

Product Code	Product Description	Unit
PROT-SIL1	ProteoSilver Stain Kit	1 kit
PROT-SIL2	ProteoSilver Plus Stain Kit	1 kit

SUGGESTED LITERATURE

P 5618	Proteomics in Practice (2002)	1 each
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