ProteoSilver™ Plus
Silver Stain Kit

Product Code **PROT-SIL2**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The ProteoSilver™ Plus Silver Stain Kit is an exceptionally sensitive, MALDI-MS compatible, protein detection silver stain kit. It is ideally suited to follow two-dimensional (2D) polyacrylamide gel analysis of complex protein solutions, a common characterization technique used in proteomics. Additionally, ProteoSilver Plus facilitates further characterization of the silver-stained protein spots, which can be destained using kit components and identified by MALDI-MS.

ProteoSilver Plus utilizes silver nitrate, which binds to selective amino acids on the proteins under weakly acidic or neutral pH conditions. The protein bound silver ions are reduced by formaldehyde at alkaline pH to form metallic silver in the gel. During destaining, the metallic silver is oxidized to silver ferricyanide, which forms a water-soluble complex when reacted with sodium thiosulfate. This kit will detect 0.1 ng of BSA/mm^2 (a protein band containing 0.2 ng of BSA on a 12 well, one-dimensional gel).

Kit Components

The ProteoSilver Plus Silver Stain Kit has sufficient components for staining 25 mini gels. The kit contains:

ProteoSilver Silver Solution (Product Code P 3739)
ProteoSilver Sensitizer (Product Code P 3614)
ProteoSilver Developer 1(Product Code P 3864)
ProteoSilver Developer 2 (Product Code P 3989)
ProteoSilver Stop Solution (Product Code P 4114)
ProteoSilver Destainer A (Product Code P 4239)
ProteoSilver Destainer B (Product Code P 4364)

Reagents and Equipment Required but not Provided

Ethanol (Product Code 27,074-1)
Acetic acid (Product Code A 9967)
Ultrapure water (16 to 18 MΩ•cm or equivalent)
Glass or plastic staining tray
Microcentrifuge tubes (for destaining gel slices)

Precautions and Disclaimer

This kit is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The use of ultrapure water is essential for low background and high sensitivity staining.

1. **Fixing solution.** Add 50 ml of ethanol and 10 ml of acetic acid to 40 ml of ultrapure water.

2. **30% Ethanol solution.** Add 30 ml of ethanol to 70 ml of ultrapure water.

3. **Sensitizer solution.** Add 1 ml of ProteoSilver Sensitizer to 99 ml of ultrapure water. The prepared solution should be used within 2 hours. A precipitate may form in the ProteoSilver Sensitizer. This precipitate will not affect the performance of the solution. Simply allow the precipitate to settle and remove 1 ml of the supernatant.

4. **Silver solution.** Add 1 ml of ProteoSilver Silver Solution to 99 ml of ultrapure water. The prepared solution should be used within 2 hours.

5. **Developer solution.** Add 5 ml ProteoSilver Developer 1 and 0.1 ml ProteoSilver Developer 2 to 95 ml of ultrapure water. The developer solution should be prepared immediately (<20 minutes) before use.

Storage/Stability

All kit components are stable at room temperature for at least 1 year.

Procedure

A. Direct Silver Staining

- All steps should be carried out at room temperature on an orbital shaker at 60 to 70 rpm.
- The gel should be stained in a glass or plastic tray, which has been cleaned with detergent and rinsed thoroughly.
- Clean, disposable gloves should be worn and changed before each step to prevent fingerprints on the gel.
- The volumes indicated in this procedure are for mini gels. The volumes should be tripled for large format (13 x 16 cm) gels.
- The staining process may be halted at the Fixing step by leaving the gel in the Fixing solution overnight if there is not enough time to complete the staining protocol.

Staining

1. Fixing - After electrophoresis of the proteins in the mini polyacrylamide gel, place the gel into a clean tray with 100 ml of the Fixing solution for 20 minutes.
   Note: A clearer background can be achieved by a longer fixing time (40 minutes to overnight).

2. Ethanol wash - Decant the Fixing solution and wash the gel for 10 minutes with 100 ml of the 30% Ethanol solution.

3. Water wash – Decant the 30% Ethanol solution and wash the gel for 10 minutes with 200 ml of ultrapure water.

4. Sensitization – Decant the water and incubate the gel for 10 minutes with 100 ml of the Sensitizer solution.

5. Water wash – Decant the Sensitizer solution and wash the gel twice, each time for 10 minutes with 200 ml of ultrapure water.

6. Silver equilibration – Decant the water and equilibrate the gel for 10 minutes with 100 ml of the Silver solution.

7. Water wash – Decant the Silver solution and wash the gel for 1 to 1.5 minutes with 200 ml of ultrapure water.
   Note: Washing for longer than 1.5 minutes will result in decreased sensitivity.

8. Gel development – Decant the water and develop the gel with 100 ml of the Developer solution. Development times of 3 to 7 minutes are sufficient to produce the desired staining intensity for most gels. Development times as long as 10 to 12 minutes may be required to detect bands or spots with very low protein concentrations (0.1 ng/mm^2).
   Note: Over development of the gel will increase the background staining.

9. Stop - Add 5 ml of the ProteoSilver Stop Solution to the developer solution to stop the developing reaction and incubate for 5 minutes. Bubbles of CO_2 gas will form in the mixture.

10. Storage – Decant the Developer/Stop solution and wash the gel for 15 minutes with 200 ml of ultrapure water. Store the gel in fresh, ultrapure water.

Destaining

1. Excise the stained spot or band of interest from the gel with a clean scalpel and place the gel slice in a clean microcentrifuge tube.
   Note: Take care to keep the excised slice intact to avoid losing gel fragments during destaining.

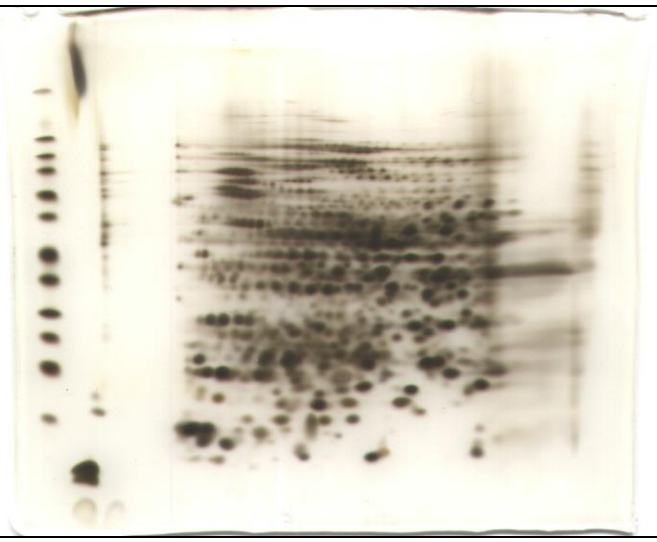
2. Add 0.1 ml of ProteoSilver Destainer A and 0.1 ml ProteoSilver Destainer B to the microcentrifuge tube. If the gel slice is not completely covered, add another 0.1 ml of ProteoSilver Destainer A and 0.1 ml ProteoSilver Destainer B to the microcentrifuge tube. Gently vortex the contents in the tube and incubate at room temperature for 2 minutes or until the stain disappears.
   Note: Do not premix Destainer solutions A and B prior to addition to the microcentrifuge tube.

3. Briefly (5 seconds) centrifuge the tube to pellet the gel slice and pipette off the supernatant.

4. Add 0.2 ml of ultrapure water to the gel slice and wash for 5 minutes. Pipette off the water and repeat this water wash twice. Gently mix, several times, during each 5 minute water wash.

5. The gel slice is now ready for further proteomic analysis, including tryptic digestion and MALDI MS. In-gel tryptic digestion may be performed using Proteomics Sequencing Grade Trypsin (Product Code T 6567).

Figure 1.
ProteoSilver Plus staining of 2D SDS-PAGE Gel.



A sample (21 µg) of lyophilized *E. coli* (Product Code EC-1) was extracted, reduced with tributylphosphine, and alkylated with iodoacetamide using the ProteoPrep® Total Extraction Sample Kit (Product Code PROT-TOT). The extract was separated by IEF on a 7 cm IPG strip (pH 4-7). The strip was transferred to a 4-12% Bis-Tris SDS-PAGE gel, with 2.5 µl of SigmaMarker (Product Code M 4038, diluted 100-fold) in the marker well. The gel was then silver stained as described in Procedure A following electrophoresis.

B. Double Staining - Silver Staining following Coomassie® Brilliant Blue Staining

Double staining (Coomassie brilliant blue and Silver) can increase the detection sensitivity 2-4 fold over that observed with silver staining alone. The gel may be initially stained using EZBlue™ Gel Staining Reagent (Product Code G 1041). The Coomassie brilliant blue stained gel must be destained until the background (gel with no protein) is essentially clear. After Coomassie brilliant blue destaining, begin the silver staining at the Fixing step (Procedure A, step 1) of the staining procedure.

References

1. Gharahdaghi, F. et al., Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: A method for the removal of silver ions to enhance sensitivity. Electrophoresis, **20**, 601-605 (1999).

2. Rabilloud, T. et al., Silver-staining of proteins in polyacrylamide gels: a general overview. Cell. Mol. Biol., **40**, 57-75 (1994).

Troubleshooting Guide

Problem	Cause	Solution
Background is excessively dark.	Gel allowed to develop too long.	Staining will be complete in less than 7 minutes. Longer staining times increase background. The intensity of an overdeveloped gel may be reduced by incubation in a 1:1 mixture of Destainer solutions A and B for less than 30 seconds, followed by extensive water washing.
	Ineffective washing due to poor mixing or improper size tray	Use proper size tray to allow the gel to be completely immersed and sufficient movement of solutions around the gel.
		Orbital shaking at 60 to 70 rpm is recommended for vigorous washing of the gel.
	Gel buffer and/or running buffer not completely removed by fixing step.	Bis-Tris buffered gels may require longer fixing time to reduce the background.
	Impure water	Use ultrapure water (16 to 18 MΩ•cm or equivalent).
Low protein sensitivity	Protein may have few cysteine residues, which are important for silver staining.	Coomassie brilliant blue staining prior to silver staining will increase silver binding to the protein (See Double Staining, Procedure B).
	Silver ions washed from the gel.	Keep the water wash time after the Silver equilibration to less than 1.5 minutes.
	Too little protein loaded on the gel.	ProteoSilver Plus will detect 0.2 ng per band of BSA. Other proteins may require higher protein loads (1 ng per band or spot).
Stained spots or blotches on the surface of the gel	Gels are not completely immersed.	Use proper size tray and sufficient mixing.
	Staining trays are not clean.	Scrub the staining tray with detergent (SigmaClean Liquid Laboratory Glassware Concentrate, Product Code S 4107) using a sponge and rinse with ultrapure water. Dedicate the tray and sponge to silver staining only.
	Fingerprints	Use disposable gloves and change them prior to each step.

Troubleshooting Guide (Continued)

Problem	Cause	Solution
Protein band around 60 to 70 kDa across the entire gel and a higher smeared background above this line or only in the lane(s) of 1D gels.	Keratin contamination from skin in the running buffer or in the sample(s)	Wear fresh, clean gloves while preparing the running buffer and do not lean over open containers. It may be necessary to make a fresh stock of running buffer or sample buffer using clean glassware.
Yellow background near the top of the gel	High concentrations of dithiothreitol (DTT) in the sample	Switch to a different reducing agent - 10 mM Tributyl phosphine (TBP - Product Code T 7567), 10 mM Tris(carboxyethyl)phosphine (TCEP - Product Code C 4706), or 100 mM 2-Mercaptoethanesulfonic acid (MESNA - Product Code M 1511).
		Alkylate the sample with a 2-5 fold molar excess of iodoacetamide and a 15 minutes incubation. Iodoacetamide will alkylate the DTT and the protein. Detection sensitivity is slightly reduced by protein alkylation.
		Use a lower concentration of DTT (30 mM) to reduce the protein sample and then dilute with sample buffer (containing 5 mM DTT) immediately before loading.
	Glycine in the Tris-Glycine-SDS running buffer can give a slight yellowing to the top portion of the gel.	Switch to a Tris-Tricine-SDS running buffer.

Checklist for ProteoSilver Staining

The following checklist can be photocopied and used to track the staining steps to ensure that all steps are carried out. Check the circle when the step is started and write the starting time.

Date_____				Date_____			
Fixing				Fixing			
	Fixing solution	20 min	O _____		Fixing solution	20 min	O _____
	Ethanol wash	10 min	O _____		Ethanol wash	10 min	O _____
	Water wash	10 min	O _____		Water wash	10 min	O _____
Sensitization				Sensitization			
	Sensitizer	10 min	O _____		Sensitizer	10 min	O _____
	Water wash	10 min	O _____		Water wash	10 min	O _____
	Water wash	10 min	O _____		Water wash	10 min	O _____
Silver Equilibration				Silver Equilibration			
	Silver solution	10 min	O _____		Silver solution	10 min	O _____
	Water wash	1 min	O _____		Water wash	1 min	O _____
Developer		3-7 min	O _____	Developer		3-7 min	O _____
Stop Solution		5 min	O _____	Stop Solution		5 min	O _____
Water wash			O	Water wash			O

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