

# **Protocol for silver staining of proteins**

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## **Introduction**

For the detection of proteins, a number of techniques have been developed in which silver staining is one of the most sensitive techniques available for visualizing the location of proteins. Each different staining technique has its own characteristics and limitations with regard to sensitivity and the type of proteins stained best. Silver staining is about 10-100 times more sensitive than various staining techniques viz. coomassie blue stain. It was introduced by Switzer *et. al* in 1979, a technique with a detection level down to 0.3-10 ng level. If one has to detect minor components then a highly sensitive detection method is needed which criterion fulfills by silver staining in both nucleic acid and protein sciences. The basic mechanism behind this method is that silver ions attached to the carboxyl and sulfhydryl groups of proteins and can be viewed after a reduction step with chemical reagents/formaldehyde. The protein bands are visualized as spots where the reduction occurs.

## **Principle**

Highly sensitive detection of proteins in the nanogram range has been achieved by the specific chemical reduction of silver ions. The silver ions form complex with amino acid side chains, carboxyl and sulfhydryl groups of proteins. Proteins are not modified. Silver ions complexed with protein are selectively reduced to metallic silver by formaldehyde. Reduction of silver by formaldehyde is concentration dependent. Formaldehyde in silver stain improves both sensitivity and contrast. Higher concentration of formaldehyde increases sensitivity but also increases background staining while higher formaldehyde level produces darker bands as opposed to the light brown bands obtained at lower concentration. Thiosulfate dissolves in soluble silver salts by complex formation removing silver ions from the gel surface which in turn decreases non specific staining. The presence of thiosulfate eliminates the formation of a dark precipitate in the gel and developer solution. The temperature of the developing solution is also play an important role. Reduction of silver is enhanced by elevated temperature of developing solution. Conversely

decreasing the temperature of the developing solution below the recommended range of 18-25° C increases the development time.

### **Reagents:**

<b><i>Fixative I:</i></b>	40% Methanol, 10% acetic acid and 0.076% formalin in double distilled water (DDW).
<b><i>Fixative II:</i></b>	10% Ethanol and 5% acetic acid in DDW.
<b><i>Oxidizer:</i></b>	3.4 mM potassium dichromate and 3.2 mM nitric acid in DDW.
<b><i>Silver reagent:</i></b>	12 mM silver nitrate (205 mg/ 100 ml) in DDW (always prepared freshly).
<b><i>Developer:</i></b>	250 mM (2.97 gm) sodium carbonate and 0.05% (50 µl) of 37% w/v formalin in DDW.
<b><i>Stopping solution:</i></b>	5% acetic acid in DDW.
<b><i>Preservative:</i></b>	30% ethanol, 10% acetic acid and 10% glycerol in DDW.

### **Procedure**

- At the end of the run, the gels were removed from the glass plates and washed in deionized water for 5 min.
- Soaked in fixative I for 1 hour. Gels can be held for several days in Fixative I.
- Soaked in fixative II for 2 hours or overnight.
- Washed in deionized water for 5 min. Thorough rinsing with water gives a low, uniform background.
- Soaked in oxidizer solution for 30 min.
- Washed 3 times in deionized water for 10 min (3 x 10 min).
- Gels were stained in a freshly made silver nitrate solution for 30 minutes.
- After staining, the gels were washed 2 times in deionized water for 4 min (2 x 4 min).
- The images were developed by soaking in developer solution for 5 to 10 min.
- When slight background staining appeared, then decants staining solution and quickly add the stop solution to stop the staining reaction. , silver stain

### **Helpful-Hints**

1. Handle the gels with gloved hands only.

2. Use deionised water.
3. Treat the glassware with concentrated nitric acid before use, it is preferable to use polyethylene zipper bags for the full procedure.
4. The reagents should be prepared when they are going to be used.
5. Quality of chemicals should be at least of analytical grade (p.a.)
6. Do not pour the staining solution directly on the gel as it may result in unequal background. Add the solution to the corner of the tray.
7. The incubations are at room temperature with shaking
8. This recipe is for one 0.75 mm thick gel; increase the quantities as per the thickness of gel increase.
9. To destain the gel put it in 3% hydrogen peroxide.

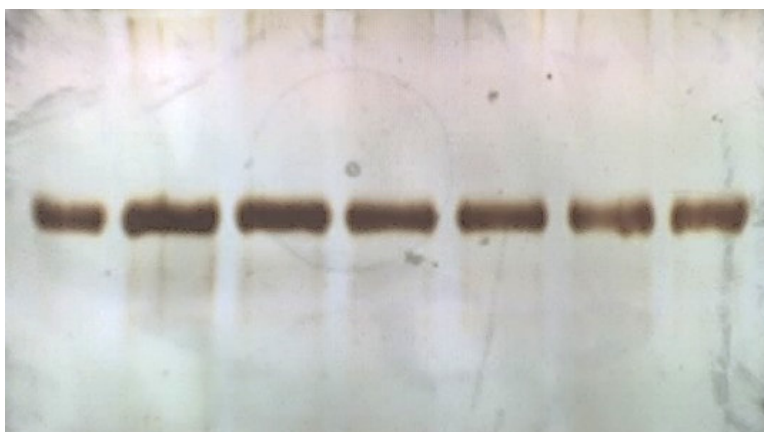


Figure.1. A well stained gel containing 10  $\mu$ g/ lane protein (Bovine serum albumin; Fraction V) on 10% polyacrylamide gel (1 mm thickness) following by this procedure.

### Reference

Switzer, R.C., 3rd, Merril, C.R., Shifrin, S. (1979). A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal Biochem.* 98(1), 231-7.