Ver.250101

PAGE Gel Silver Staining Kit

G7210

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Introduction

In the mid-17th century it was discovered that silver has ability to develop images. This property of silver was exploited in the development of photography and in several histological procedures. Silver staining for detection of proteins following gel electrophoresis was first reported in 1979 by Switzer et al. resulting in a major increase in the sensitivity of protein detection. This staining method is generally accepted to be several times more sensitive than methods using Coomassie Brilliant Blue R-250.

All silver staining methods depend upon the reduction of ionic silver to its metallic form. For increase in sensitivity the gel is treated with reducing or sulfiding agents like Glutaraldehyde, Formaldehyde and Sodium thiosulphate which has affinity for proteins prior to silver impregnation. Glutaraldehyde forms a Schiff's base with the proteins which results in the cross linking of amine groups and aldehydes which reduces silver during development. Protein bands can be visualized on the gel due to the difference in oxidation/reduction potential between the sites occupied by protein and the adjacent sites not occupied by the protein on the gel. The silver cations complex with protein amino groups, particularly the amino group of lysine, and with sulphur residues of cysteine and methionine. The gel is impregnated with silver nitrate in a weakly acidic (pH 6) solution. Any free silver nitrate is washed out of the gel prior to development, as precipitation of silver oxide will result in high background staining. Development is subsequently achieved by selective reduction of ionic silver by formaldehyde made alkaline by sodium carbonate. Sodium carbonate acts as a buffer to the formic acid produced by the oxidation of formaldehyde, which results in reduction of silver ion till protein bands appear in the gel.

Kit components

Reagent	Volume	Storage
1.2% Glutaraldehyde	100 mL	2 – 8°C
0.5%Sodium thiosulphate	100 mL	2 – 8°C
2.5% Silver nitrate	100 mL	2 – 8°C
0.19% Formaldehyde	2×100 mL	2 – 8°C
Sodium carbonate	50g	2 – 8°C

Materials needed but not provided

- Ethanol
- Molecular Biology Grade Water
- Acetic acid

Preparation of Reagents (100 mL)

- Fixing Solution:

  Ethanol……40mL
  Acetic acid……..10 mL
  Distilled water…….50 mL

- Fix/Sensitizing Solution:
  Ethanol……40 mL
  1.2% Glutaraldehyde……...4 mL
  0.19% Formaldehyde……..2 mL
  Distilled water…….54 mL

- Ethanol Rinsing Solution:
  Ethanol……..40 mL
  Distilled water…….60 mL

- Sensitizing Solution:
  0.5% Sodium thiosulphate….4 mL
  Distilled water……...96 mL

- Silver Solution:
  2.5% Silver nitrate…… 4 mL
  Distilled water……96 mL

- Developing Solution:
  Sodium carbonate……....2.5 g
  0.19% Formaldehyde……..8 mL
  Distilled water……92 mL

- Stop Solution:
  Acetic acid……..5 mL
  Distilled water……95 mL

Procedure

- After completion of SDS-PAGE, carefully remove the gel from in-between the glass plates into a clean tray. Add 50 mL of Fixing solution and incubate for 10 minutes with constant moderate shaking.
- Discard the Fixative Solution. Wash with distilled water for 10 minutes.
- Add 50 mL of Fix/Sensitizing Solution and shake for 5 minutes.
- Discard the Fix/Sensitizing Solution. Wash the gel with 40% Ethanol for 20 minutes.
- Wash the gel with distilled water for 20 minutes.
- Add Sensitizing Solution and shake for one minute.
- Discard the Sensitizing Solution and wash the gel twice with distilled water for one minute.
- Incubate the gel in 50mL of Silver Solution for 20 minutes ensuring that the gel is completely submerged in the liquid. Incubate with constant moderate shaking.
- Remove the gel from the Staining Solution and wash it with distilled water for one minute.
- Add the Developing solution and shake for 1minute. Remove this solution and add fresh. Incubate the gel in Developing Solution until color of separated protein bands develop and are distinctly visible.
- Remove the gel from the Developing Solution and immerse the gel in Stopping Solution for 5 minutes to obtain sharp distinct bands.
- Discard the Stop Solution and wash the gel with distilled water 2 times for 5 minutes.

Troubleshooting Guide

SL. No	Problem	Possible Cause	Solution
1	Gray or brown precipitate appears as smudges	Non-specific deposition of silver	Increase the washing steps.
2	Large discolored spots on the gel	Pressure on the gel during handling	Keep minimal contact with the gel while performing the protocol.
3	Slow or no development of bands	Developer solution is not mixed properly	Ensure the Developing Solution is mixed thoroughly before use
4	Low band sensitivity	Long exposure of Reducer Solution	Do not place the gel in reducer solution for more than 1 minute

Safety Information

The PAGE Gel Silver Staining Kit is for laboratory use only, not for drug, household or other uses. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

Performance of the kit is expected when the kit is stored at recommended temperature and within the expiry period.

Product Use Limitation & Warranty

Silver Staining Kit is designed and sold for research and in vitro purposes only. The product is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressed clearly for that purpose by the Food and Drug Administration or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in the text.