CREATIVE BIOMART®
Assay Kit

Rapid Silver Stain Kit

Product Information

Cat

Kit-2165

Common Name

Silver Stain

Cat.No. Kit-2165

Product Overview

Silver Stain Kit is an exceptionally sensitive protein detection silver stain kit. It is suitable for staining both single dimension SDS-PAGE gels and two-dimensional (2D) gels of complex protein solutions, a common characterization technique used in proteomics.

Description

Silver Stain Kit 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.

Notes

· 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.

Storage

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

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Synonyms

Silver Stain Kit; Fast Silver Stain Kit

Size

25 mini gels

Kit Components

Silver solution
Silver Stain Sensitizer
Silver Stain Developer 1
Silver Stain Developer 2
Stop Solution

Materials Required but Not Supplied

Ethanol
Acetic acid
Ultrapure water
Glass or plastic staining tray

Preparation

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 Silver Stain Sensitizer to 99 ml of ultrapure water. The prepared solution should be used within 2 hours. A precipitate may form in the Silver Stain 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 Silver Solution to 99 ml of ultrapure water. The prepared solution should be used within 2 hours.
5. Developer solution. Add 20 ml Silver Stain Developer 1 and 0.05 ml Silver Stain Developer 2 to 80 ml of ultrapure water. The developer solution should be prepared immediately (<20 minutes) before use.

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Assay Protocol

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 2 minutes with 100 ml of the Sensitizer solution.
5. Water wash – Decant the Sensitizer solution and wash the gel twice, each time for 1 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 100 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.
Note: Over development of the gel will increase the background staining.
9. Stop - Add 5 ml of the 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 2-5 minutes with 100 ml of ultrapure water. Store the gel in fresh, ultrapure water.

Sensitivity

0.3ng BSA protein

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