FireSilver-Staining-Protocol

Materials

Kit Contents (Stock Solutions, store at 4°C)

20 mg Sensitizer 1 (10 x)

1 x Sensitizer 2 (10 ml)

100 x Silver Solution (10 ml)

5 x Pre-Developer Solution (200 ml)

5 x Developer 1 Solution (200 ml)

100 x Developer 2 Solution (10 ml)

5 x Stop Solution (200 ml)

Staining Protocol

Additional Required Materials

96% Ethanol

Acetic acid

Ultra pure water

Prepare solutions A-G (store at 4°C, prepare fresh for best results)

A **Fixing solution**: 50% Ethanol, 10% Acetic Acid in pure water (25 ml Ethanol, 5 ml Acetic Acid adjust to 50 ml with water).

B **Ethanol Wash solution**: 20% Ethanol (10 ml Ethanol adjust to 50 ml with water).

C **Sensitizer solution**: Add 1ml **Sensitizer 2** to 20mg **Sensitizer 1** (use solution not longer than one week!), dilute this solution before use 1:100 with ultra pure water, (500µl - adjust to 50 ml with water).

D **Silver solution**: Dilute the **Silver Stock Solution** 1:100 with ultra pure water before use (500µl - adjust to 50 ml with water).

E **Pre-developer solution**: Dilute the **Pre-Developer Stock Solution** 1:5 with ultra pure water before use (10 ml - adjust to 50 ml with water).

F **Developer solution**: Dilute the **Developer 1 Stock Solution** 1:5 with ultra pure water and pipette 1/100 volume **Developer 2 Stock Solution** to the mixture (10 ml Developer stock solution 1 - adjust to 50 ml with water plus 500µl Developer Stock Solution 2).

G **Stop solution**: Dilute the **Stop Solution** 1:5 with ultra pure water before use (10 ml - adjust to 50 ml with water).

Staining procedure

1. Fixing - After electrophoresis place the gel (7x8 cm) into a clean tray with 50 ml of the Fixing solution **A** at least for 30 minutes up to over night.

   Note: A Longer fixing period increase the sensitivity.

2. Ethanol wash - Decant the Fixing solution and wash the gel for 10 minutes with 50 ml of the 20% Ethanol solution **B**.

3. Sensitization – Incubate the gel for 1 minute with 50 ml of the Sensitizer solution **C**.

4. Water wash 1 – Wash the gel for 1 minute with 100 ml of ultra pure water.

5. Water wash 2 – Repeat step 4.

6. Silver equilibration – Equilibrate the gel for 30 minutes with 50 ml of the Silver solution **D**.

7. Water wash 3 – Wash the gel for 30 seconds with 50 ml of ultra pure water.

   Note: Washing longer than 1.5 minutes will result in decreased sensitivity.

8. Gel pre-development – Pre-develop the gel for 1 minute with 50 ml of the Pre-developer solution **E**.

   Note: Incubation longer than 2 minutes may results in increased background.

9. Gel development – Develop the gel for 1-15 minutes with 50 ml of the Developer solution **F***.

   Note: Development times longer as 15 minutes may be required to detect bands or spots with very low protein concentrations (< 1 ng/mm^2).

10. Stop – Incubate the gel for 20 minutes in Stop solution **G**.

11. Storage – Store the gel in ultra pure water.

Note: Shake the gel during the process gently.

*Waste disposal: 9, R: 26/27/28-33-50/53, S: 13-28.1-36-45-60-61 / WGK: 3