CHAPTER FOURTEEN

Silver Staining of SDS-polyacrylamide Gel

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Abstract

To detect nanogram quantities of protein and nucleic acids on SDS-PAGE gels.

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1. THEORY

Silver staining is more sensitive than both Coomassie blue (see Coomassie Blue Staining) and ethidium bromide staining (see Agarose Gel Electrophoresis). The chemistry of silver staining relies on the reduction of silver from an ionic state to a metallic state. Macromolecular bands are stained silver on a clear background. In practice, silver staining allows you to detect smaller amounts of protein and to easily check the purity of your sample.

2. EQUIPMENT

Platform rotator
Plastic gel staining box
Gloves
Gel documentation system
Gel dryer or gel drying rack

3. MATERIALS

Nitric acid (HNO_3)
Ethanol, 95%
Glacial acetic acid
Glycerol
Methanol (MeOH)
Formaldehyde, 37% (Sigma)
Sodium thiosulfate ($Na_2S_2O_3$)
Sodium carbonate (Na_2CO_3, anhydrous, ACS grade)
Silver Nitrate ($AgNO_3$)

3.1. Solutions & buffers

Step 1 Fixing solution

Component	Final concentration	Stock	Amount
Methanol	50%	100%	50 ml
Acetic acid	12%	100%	12 ml
Formaldehyde	0.0185%	37%	50 µl

Add water to 100 ml. Note: add formaldehyde immediately before use

Wash buffer

Mix 250 ml methanol with 250 ml water to give a final concentration of 50%

Step 2 Sodium thiosulfate solution

Dissolve 50 mg sodium thiosulfate, $Na_2S_2O_3$, in 250 ml water to give a concentration of 1.3 mM. Make fresh

Silver solution

Component	Final concentration	Stock	Amount
$AgNO_3$	11.8 mM	N/A	0.2 g
Formaldehyde	0.028%	37%	75 µl

Add water to 100 ml purified water. Make fresh

Developing solution

Component	Final concentration	Stock	Amount
Na_2CO_3 (anhydrous)	6% (w/v)	N/A	12 g
Sodium thiosulfate solution	0.05 mM	1.3 mM	4 ml
Formaldehyde	0.037%	37%	100 µl

Add water to 200 ml. Make fresh

Stop solution

Component	Final concentration	Stock	Amount
Methanol	50%	100%	50 ml
Acetic acid	12%	100%	12 ml

Add water to 100 ml

Step 3 Wash buffer 2

Dilute 30 ml methanol in 70 ml water to give a final concentration of 30%

Storage solution

Component	Final concentration	Stock	Amount
Ethanol	20%	100%	20 ml
Glycerol	10%	100%	10 ml

Add water to 100 ml

4. PROTOCOL

4.1. Duration

Preparation	1 h
Protocol	4 h

4.2. Preparation

Run SDS-PAGE mini-gel (see One-dimensional SDS-Polyacrylamide Gel Electrophoresis (1D SDS-PAGE)) and place in a clean gel staining box.

4.3. Tip

Gel boxes should be cleaned with 50% nitric acid and then rinsed thoroughly with water.

4.4. Tip

Handle gels delicately. Wear gloves to avoid transferring fingerprints and avoid manipulating it with metal tools such as tweezers.

4.5. Tip

Staining will be uneven if the gel is not completely submerged in liquid. Increase buffer volumes as appropriate.

4.6. Tip

Times and volumes are appropriate for mini-gels. Increase both for thicker gels.

See Fig. 14.1 for the flowchart of the complete protocol.

5. STEP 1 FIX THE GEL

5.1. Overview

The proteins are immobilized and the SDS-PAGE running buffer is removed.

5.2. Duration

2.5 h

1.1 Add 50 ml water to the gel in small container, swirl gently for 5 s, and decant water.

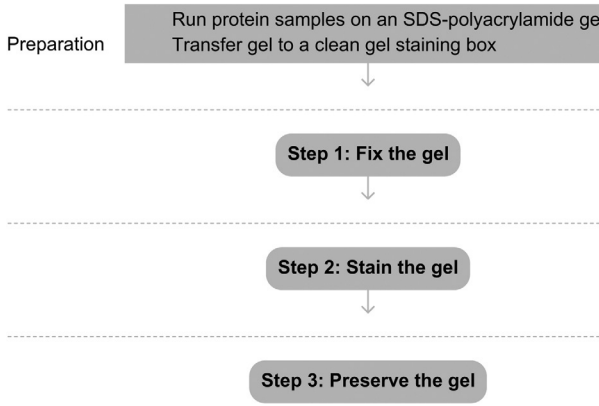

Figure 14.1 Flowchart of complete protocol, including preparation.

1.2 Repeat Step 1.1 two to four more times.
1.3 Add 50 ml of Fixing Solution to the gel. Rotate the gel for at least 1 h. Carefully decant Fixing Solution.
1.4 Add 50 ml of Wash Buffer. Rotate for 20 min. Decant buffer.
1.5 Repeat Step 1.4 two more times.
1.6 Add 50 ml water to the gel in small container, swirl gently for 5 s, and decant water.
1.7 Repeat Step 1.6 two to four more times.

5.3. Tip

The g*el can be left overnight in Fixing Solution.*

See Fig. 14.2 for the flowchart of Step 1.

6. STEP 2 STAIN THE GEL

6.1. Overview

The gel is incubated first in a silver solution and then in the developing solution, causing the silver to precipitate onto the protein.

6.2. Duration

1 h

2.1 Add 5 ml of sodium thiosulfate solution and incubate rotating for 1 min.

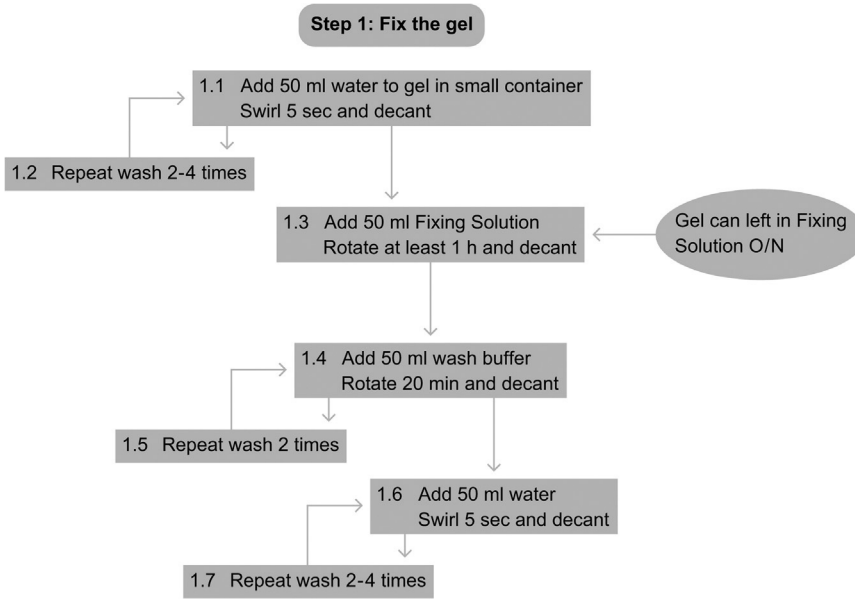

Figure 14.2 Flowchart of Step 1.

2.2 Add 50 ml water to the gel in small container, swirl gently for 5 s, and decant water.
2.3 Repeat Step 2.2 two to four more times.
2.4 Add 50 ml of Silver Solution. Incubate rotating for 20 min. Decant solution.
2.5 Add 50 ml water to the gel in small container, swirl gently for 5 s, and decant water.
2.6 Repeat Step 2.5 two to four more times.
2.7 Add 50 ml of Developing Solution. Quickly swirl in the gel box and decant.
2.8 Add 50 ml of Developing Solution. Incubate rotating until desired staining intensity is achieved.
2.9 Add 50 ml water to the gel in small container, swirl gently for 5 s, and decant water.
2.10 Repeat Step 2.9 two to four more times.
2.11 Add 50 ml of Stop Solution.
2.12 Photograph the gel using a gel documentation system.

See Fig. 14.3 for the flowchart of Step 2.

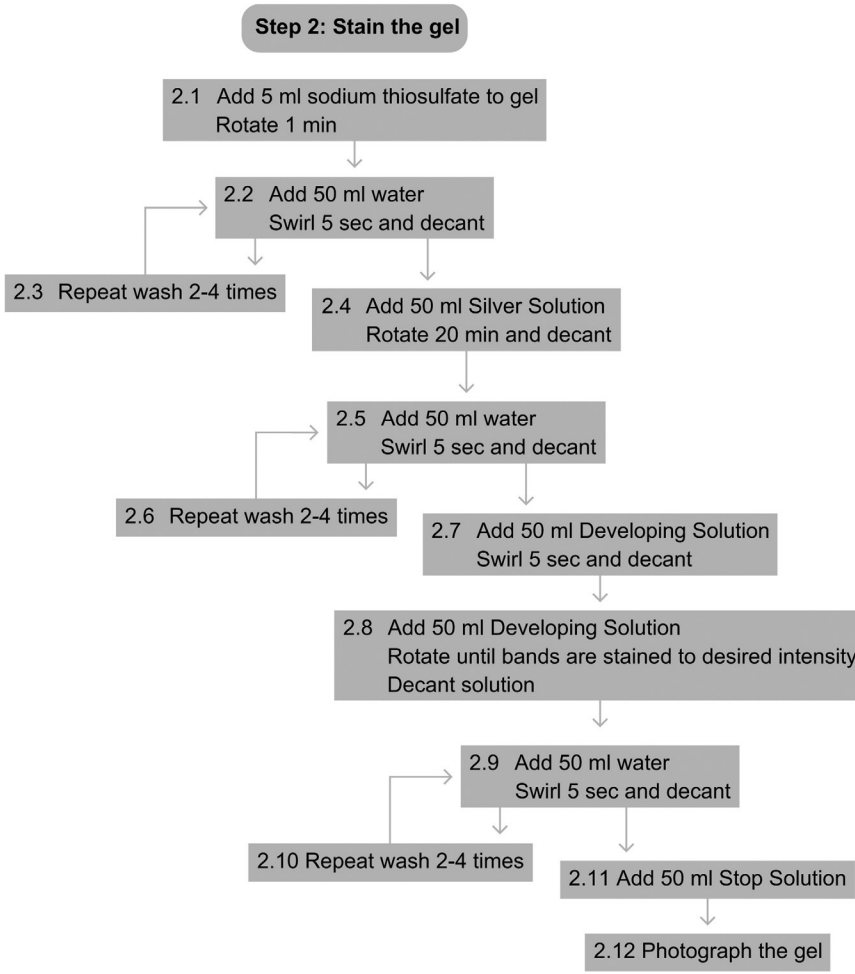

Figure 14.3 Flowchart of Step 2.

7. STEP 3 PRESERVE THE GEL

7.1. Overview

The gel is washed into a buffer for long-term storage.

7.2. Duration

45 min

3.1 Decant the Stop Solution.

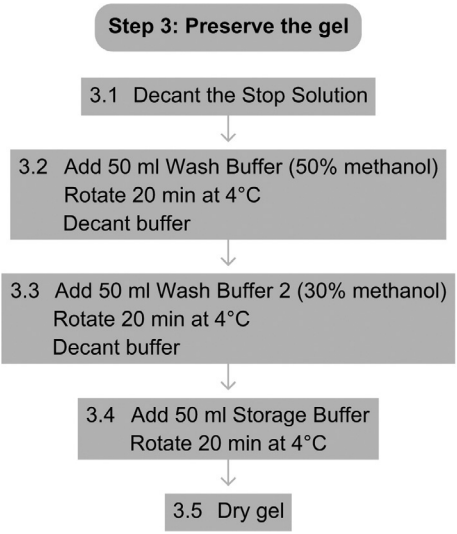

Figure 14.4 Flowchart of Step 3.

3.2 Add 50 ml of Wash Buffer (50% methanol). Incubate rotating for 20 min at 4 °C. Decant buffer.
3.3 Add 50 ml of Wash Buffer 2 (30% methanol). Incubate rotating for 20 min at 4 °C. Decant buffer.
3.4 Add 50 ml of Storage Solution. Incubate rotating for 20 min at 4 °C.
3.5 The gel can now be dried using your preferred method.
See Fig. 14.4 for the flowchart of Step 3.

REFERENCES

Referenced Protocols in Methods Navigator
Coomassie Blue Staining.
Agarose Gel Electrophoresis.
One-dimensional SDS-Polyacrylamide Gel Electrophoresis (1D SDS-PAGE).