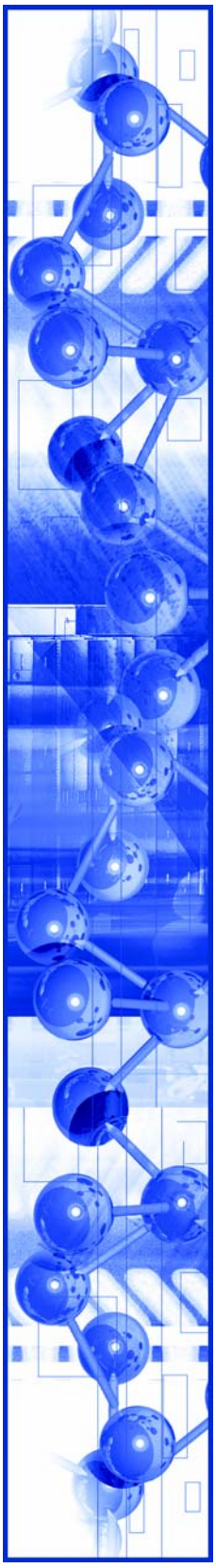

# Silver BULLit™ Silver Stain Kit

| Code | Description | Size |
|---|---|---|
| M227-1L-KIT | Silver BULLlit™ Silver Stain Kit<br>*Includes*:<br>Sensitizer 10X, 250 ml<br>Silver Stain Solution 10X, 250 ml<br>Developer 5X, 250 ml x 2<br>*Sufficient to stain 25-50 mini-gels, 10 cm x 10 cm x 0.75* | 1 Kit |
| M227-Q-KIT | Silver BULLit™ Silver Stain Kit<br>*Includes*:<br>Sensitizer 10X, 50 ml<br>Silver Stain Solution 10X, 50 ml<br>Developer 5X, 50 ml x 2<br>*Sufficient to stain 5-10 mini-gels, 10 cm x 10 cm x 0.75* | 1 Sample Kit |

**General Information:**

Silver BULLit™ Silver Stain Kit is an extremely sensitive colorimetric staining procedure for the detection of subnanogram amounts of proteins resolved on polyacrylamide gels. With high sensitivity and very low background, Silver BULLit™ is ideal for the visualization of protein bands in dilute samples or for the detection of proteins present in trace amounts. It is generally about 100 fold more sensitive than the commonly used Coomassie® Blue stain[1].

Two procedures are included with the Silver BULLit™ Silver Staining Kit. The original staining procedure, which takes 4 hours, is recommended for maximum sensitivity. The 30 minute fast protocol, useful for situations when rapid results are required, is optimized for 0.75 mm mini-gel formats. Protocols are similar except for the length of time for each step. Both begin with a fixation step to reduce band diffusion and remove interfering substances. A rapid sensitization process which enhances binding of silver ions to proteins follows fixation. The staining step allows silver ions to permeate the gel and bind preferentially to sulfhydryl and carboxyl side chains. In the final development step, silver ions are reduced to metallic silver and form precipitates that are visible as brown bands within the gel[2].

Staining intensity varies by the type of protein in the sample. Acidic proteins and proteins with highly negatively charged sulfated sugar residues such as proteoglycans and mucins are not readily detected by silver stains.

**Storage:**

Store product at room temperature.

**Application Disclaimer**

*For Research Use Only.*
*Not for Therapeutic or Diagnostic Use.*

## Protocol:

**Reagents supplied in Kit:**

- 10X Sensitizer Solution (250 ml)
- 10X Silver Stain Solution (250 ml)
- 5X Developer Solution (250 ml x 2)

**Required Reagents not included in Kit.**

- Methanol
- Ethanol
- 37% Formaldehyde
- Glacial Acetic Acid
- Distilled/Deionized Water

**Note:**

- **All incubations should be conducted with continuous agitation on a shaker.**
- Solutions should be freshly prepared on day of use.
- All steps should be performed at room temperature.
- Water should be deionized and distilled.
- Staining should be carried out in clean glass containers with sufficient quantities of solution to immerse the gel and allow it to move freely during agitation. Use separate containers for each gel.
- Powder-free gloves should be worn during the procedure. Do not touch gel plates, staining dishes or gels with bare hands as prints will be visible on the stained gels. Rinse gloves in water between each step.

**Note:**

- If crystals form in the developer solution during storage, warm gently until they re-dissolve. The 10X Silver Stain Solution may appear cloudy or contain a fine precipitate. This appearance will not affect the performance of the stain.

## Original Procedure – Maximum Sensitivity (4 hours)

**Solutions**:

**Fixative** (100 ml)

| Component | Volume |
|---|---|
| Methanol | 50.00 ml |
| Glacial Acetic Acid | 12.00 ml |
| 37% Formaldehyde | 2.35 ml |
| Distilled/Deionized Water | 37.00 ml |

**35% Ethanol** (300 ml)

| Component | Volume |
|---|---|
| 95% Ethanol | 81.00 ml |
| Distilled/Deionized Water | 219.00 ml |

**1X Sensitizer Solution** (100 ml)

| Component | Volume |
|---|---|
| 10X Sensitizer Solution | 10.00 ml |
| Distilled/Deionized Water | 90.00 ml |

**1X Stain Solution** (100 ml)

| Component | Volume |
|---|---|
| 10X Stain Solution | 10.00 ml |
| Distilled/Deionized Water | 90.00 ml |

**1X Developer Solution** (100 ml)

| Component | Volume |
|---|---|
| 5X Developer Solution | 20.00 ml |
| Distilled/Deionized Water | 80.00 ml |

**Stop Solution** (100 ml)

| Component | Volume |
|---|---|
| Methanol | 50.00 ml |
| Glacial Acetic Acid | 12.00 ml |
| Distilled/Deionized Water | 38.00 ml |

1. Fix gel in 100 ml of fixative 2 hours to overnight.
2. Wash 3 times for 20 minutes each in 35% Ethanol.
3. Incubate gel in 100 ml Sensitizer Solution for 2 minutes.
4. Wash 3 times for 5 minutes each in distilled/deionized water.
5. Incubate gel for 20 minutes in Stain Solution.
6. Wash gel in distilled/deionized water 2X for 1 minute each.

7. Incubate gel in Developer Solution until bands become visible (approximately 10 minutes for full development). Bands should appear dark brown against a pale background.

**Note:**

- The rate of band development is temperature dependent.
- If the gel is over-developed, artifacts are present, the background is too dark, or if the bands are over-stained, the gel can be de-stained with AMRESCO® Silver Subtract (M322-Kit) and restained as desired.

8. Incubate for 20 minutes in Stop Solution to prevent further color development.
9. Store at 4°C in 1% Acetic Acid.
10. Gels may be photographed on a bright white light box.

## FAST Silver Stain Procedure using Silver BULLit™ Kit: (30 minutes)

**Note:** This procedure is recommended for 0.75 mini-gel formats.

**Solutions**:

**Fixative-1** (100 ml)

| Component | Volume |
|---|---|
| Methanol | 35.00 ml |
| Glacial Acetic Acid | 10.00 ml |
| 37% Formaldehyde | 2.70 ml |
| Distilled/Deionized Water | 52.30 ml |

**Fixative-2** (100 ml)

| Component | Volume |
|---|---|
| Ethanol | 35.00 ml |
| Glacial Acetic Acid | 10.00 ml |
| 37% Formaldehyde | 2.70 ml |
| Distilled/Deionized Water | 52.30 ml |

**1X Sensitizer Solution** (100 ml)

| Component | Volume |
|---|---|
| 10X Sensitizer Solution | 10.00 ml |
| Distilled/Deionized Water | 90.00 ml |

**1X Stain Solution** (100 ml)

| Component | Volume |
|---|---|
| 10X Stain Solution | 10.00 ml |
| Distilled/Deionized Water | 90.00 ml |

**1X Developer Solution** (100 ml)

| Component | Volume |
|---|---|
| 5X Developer Solution | 20.00 ml |
| Distilled/Deionized Water | 80.00 ml |

**1X Stop Solution** (100 ml)

| Component | Volume |
|---|---|
| Methanol | 50.00 ml |
| Glacial Acetic Acid | 10.00 ml |
| Distilled/Deionized Water | 40.00 ml |

1. Incubate gel in 100 ml of Fixative-1 for 5 minutes with agitation. Gels can be left in Fixative-1 over night if desired.
2. Incubate gel in 100 ml of Fixative-2 for 5 minutes with agitation.
3. Incubate gel in 100 ml of 1X Sensitizer for 2 minutes.

4. Rinse gel 3 times in large volumes of distilled/deionized water.
5. Incubate gel in 100 ml of 1X Silver Stain for 10 minutes.
6. Rinse gel 3 times with distilled/deionized water.
7. Incubate gel in 100 ml of 1X Developer for 5 minutes or until desired band intensity is obtained.
8. Place gel in 1X Stop Solution to prevent further band development.
9. Store gel at 4$^{o}$C in 1% Acetic Acid.
10. Gels may be photographed on a bright white light box.

**Tips:**

- Double the times for Fix-1 and Fix-2 to reduce background staining.
- To increase sensitivity, develop longer time periods.
- Dispose of AgNO3 appropriately.
- Gel can be left in Fix-1 solution overnight.

**Troubleshooting:**

Background is too dark:

Residual acid in gel
- Increase washing time after the fixation step.

Poor quality acrylamide
- Acrylamide quality can affect the background appearance of a silver-stained gel. Use ultrapure grade acrylamide such as AMRESCO's Acryl/Bis™ 37.5:1, 40% Solution (W/V), Code:0254.

Poor quality water
- Use deionized/distilled water in all solutions

Negative staining

Excess protein in bands.
- Reduce the amount of protein applied to gel.

Streaking or yellow background

Excess reducing agents such as 2-mercaptoethanol or DTT.
- Reduce the amount of reducing agent in sample buffer.

Artificial bands with apparent molecular weights between 50-70 kDa

Excess amounts of reducing agents such as 2-mercaptoethanol or DTT.
- Lower the amount of reducing agent in sample buffer.

**References:**

1. Merril, C.R. et. Al., Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. *Proc Natl Acad Sci USA.* 1979. 76:4335-4339.

2. Merril, C.R., et. al., Simplified silver protein detection and image enhancement methods in polyacrylamide gels. *Electrophoresis.* 1982. 3: 17-23.

## Related Products

| Code | Product |
|---|---|
| M322-Kit | Silver Subtract™<br>Silver Stain De-Stain |
| M256-100ML | NEXT GEL™ 10%: Premixed Acrylamide Solution and Running Buffer for SDS-PAGE |
| M260-5.0ML | NEXT GEL™ Sample Loading Buffer, 4X |
| See our catalog for a complete list of NEXT GEL™ products | |
| 0254-500ML | Acryl/Bis™ 37.5:1, 40% Solution (W/V) |
| 0783-4L | Tris-Glycine-SDS Buffer, Liquid Concentrate, 10X |
| J383-200UL | Precise™ Protein Molecular Weight Marker, 7 bands, 15.0-150.0 kDa |

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