In-gel protein staining with silver nitrate

Silver staining detects proteins after electrophoretic separation with high sensitivity, down to the sub-nanogram range per band or spot. Unlike dyes, which require a minimum mass of protein to give detectable signal, silver staining amplifies even tiny amounts via autocatalytic silver reduction at protein-bound sites, particularly at thiols and other reactive side chains, which nucleate the formation of visible metallic silver.

This protocol describes the classical silver nitrate method (Blum et al., 1987; Chevallet et al., 2006), which uses formaldehyde-based image development in alkaline carbonate. Compared to other silver stains—such as silver-ammonia (Rabilloud, 1990) or variants optimized for mass-spectrometry (Shevchenko et al., 1996)—this method is simple, robust, and inexpensive, making it particularly suited for analyzing many samples in parallel. However, the resulting signal is nonlinear and less compatible with downstream applications like mass spectrometry, immunodetection, or enzymatic activity assays. In those cases, Coomassie SOP 0010 or fluorescent stains may offer better quantification or functional recovery.

Risk assessment

- **Formaldehyde is a KNOWN CARCINOGEN**
- Silver nitrate is a STRONG OXIDANT; keep away from combustible materials
- Silver nitrate causes severe skin burns and eye damage

▷ Wear gloves, safety glasses, lab coat

- ☐ Collect formaldehyde solutions as HAZARDOUS WASTE
- ☐ Collect silver containing solutions as SILVER WASTE
- ☐ Precipitate silver nitrate solutions with sodium chloride or ascorbic acid
- ☐ DO NOT wash into sewer



Reviewed: Nov 2, 2026

Procedures

›› *Protein fixation*

☐ Fixation solution R0170 ☐ 30% Ethanol

(1.) Fix the gel in 100–200 mL fixation solution three times for 30 min each on a rocking table. 90 min

> ***Critical:*** Do not use aldehydes if mass spectrometry is planned. Fixation with methanol and acetic acid is sufficient, but aldehydes markedly improve sensitivity and uniformity.

> ***Hint:*** A single 30 min fixation improves sequence coverage in mass spectrometry, but bands turn yellow to brown, which precludes image analysis; fixing three times for 30 min each gives better staining. Fix 2D gels for at least 18 h to remove carrier ampholytes.

> ***Hint:*** Use at least 5-fold excess of fixation solution over gel volume. Multiple gels can be stained at once, but must float freely in the solution.

(2.) Wash the gel twice in 30% ethanol for 10 min each. 20 min

(3.) Wash the gel twice in water for 10 min each. 20 min

›› *Sensitization and impregnation with silver nitrate*

- ☐ Sodium thiosulfate sensitizing solution ◇ R0126
  - *or*: Tetrathionate sensitizing solution ◇ R0127
- ☐ 0.2% Silver nitrate ◇ R0128

(1.) Prepare four containers: one for the sensitization solution, two for rinsing with water, and one for the silver nitrate impregnation. Gels should move sequentially through each solution without delay.

(2.) Sensitize by soaking one gel at a time in one of the following:

- ○ For a fast, sensitive staining, incubate for 1 min in *sodium thiosulfate* sensitizing solution.
- ○ For consistent, linear staining, incubate for 45 min in *tetrathionate* sensitizing solution.

(3.) Rinse the gel twice in water for 1 min each after thiosulfate. After tetrathionate, rinse twice in 20% ethanol for 10 min each, then four times in water for 10 min each. Proceed immediately to silver impregnation.

(4.) Impregnate with silver nitrate for 20–120 min.

Hint: Gels may turn yellow. The duration of impregnation does not strongly affect the final image quality.

›› *Image development*

- ☐ Silver nitrate developer ◇ R0129
- ☐ Tris stop solution ◇ R0264

(1.) Wash one gel at a time by briefly dipping in water.

Hint: Prepare one box half-filled with water, one developer box per gel, and a shared stop solution box. Transfer gels along this line. Only one gel should be in the developer at a time.

(2.) Transfer gel into developer. Shake until precipitate dissolves.

Critical: A brown or gray precipitate typically appears within seconds. Shake immediately to redissolve it or risk particulate background.

(3.) When desired degree of staining is achieved, stop the reaction by transferring gel into Tris stop solution. Incubate for at least 30 min.

Quality assurance: Intense bands or spots may appear a few minutes into development. Allow development to proceed until background is acceptable. No further changes will occur beyond 45 min. Aim to stop development when protein bands are clearly visible against a faint, uniform yellow-to-brown background. If the background darkens to a deep brown before bands are clearly resolved, the sensitization or impregnation step may need adjustment.

Note: Multiple gels can be stacked in the same stop solution box at this stage.

(4.) Wash gels at least twice for 30 min in water. 60 min

Storage of stained gels

- ☐ 10% Glycerol

(1.) Leave stained gels in water for up to several days.

Critical: For mass spectrometry, staining, washing, spot excision, and destaining should be performed on the same day.

(2.) For permanent storage, soak the gel in 10% glycerol for at least 20 min.

This is why: This prevents cracking during drying and helps preserve band integrity for documentation.

Destaining for mass spectrometry

☐ 200 mM Ammonium hydrogencarbonate R0201	☐ Silver nitrate destaining solution R0171

(1.) Excise the band or spot of interest from the gel.

(2.) Cover the gel slice with 150 µL destaining solution and incubate for 5–10 min. Remove the solution. 5–10 min

(3.) Rinse the gel slice five times with 150 µL water for 5 min each. Remove the water between rinses.

(4.) Soak the gel slice in 200 mM ammonium hydrogencarbonate solution for 20 min. 20 min

(5.) Rinse again with water as before.

(6.) Proceed to in-gel digestion, or dry and store gel pieces at −20 °C until use.

> ***Note:*** If possible, proceed immediately to in-gel digestion to minimize protein oxidation.

[GWW+99]

Troubleshooting

Sensitization and impregnation with silver nitrate

In Step 4:

- Metallic silver deposits on the gel surface (silver mirror)
  - Use clean, grease-free glass plates when casting gels.
  - Avoid handling gels with bare or powdered gloves; always use powder-free gloves and minimize direct contact.

Image development

In Step 2:

- Development does not take place or yields only a weak image
  - Silver solution was incorrectly diluted.
  - Potassium bicarbonate was mistakenly used instead of potassium carbonate.
  - For precious samples: stop the reaction, rinse thoroughly with water, ten times for 30 min each, and restart from the beginning.
- Strong yellow background.
  - Thiosulfate is exhausted or has decomposed. Replace with a fresh supply.
- Developer turns black; deposits fail to dissolve.
  - Likely thiosulfate exhaustion. Use fresh thiosulfate stock.

Materials

Protein fixation

- ☐ **Fixation solution** R0170
- ☐ **Ethanol**, 30%
  CAS 64-17-5

Sensitization and impregnation with silver nitrate

- ☐ **Sensitizing solution**
  - **Sodium thiosulfate sensitizing solution** R0126
  - *or* **Tetrathionate sensitizing solution** R0127
    Stable for 1 day at room temperature.
- ☐ **Silver nitrate**, 0.2% R0128
  Store at room temperature, shielded from light.

Image development

- ☐ **Silver nitrate developer** R0129
  Stable for 1 day at room temperature.
- ☐ **Tris stop solution** R0264
  Stable for 1 day at room temperature.

Storage of stained gels

- ☐ **Glycerol**, 10%
  CAS 56-81-5

Destaining for mass spectrometry

- ☐ **Ammonium hydrogencarbonate**, 200 mM R0201
- ☐ **Silver nitrate destaining solution** R0171

Recipes

Fixation solution

Amount	Ingredient		Stock	Final
60 mL	Ethanol, non-denatured Flammable liquid (H225)	[64-17-5]	100%	30%
20 mL	Acetic acid, glacial Skin corrosion (H314)	[64-19-7]	99.6%	10%
To 200 mL	Water, reagent-grade			

Fixation solution

30% Ethanol, 10% Acetic acid



DANGER

Flammable liquid; Skin and serious eye irritation

- ☐ Collect as ORGANIC SOLVENT WASTE
- ☐ Keep away from oxidizers

Date: Sign: R0170

Sodium thiosulfate, 640 mM

Amount	Ingredient		Stock	Final
1.01 g	Sodium thiosulfate	[7772-98-7]	158.11 g/mol	640 mM
To 10 mL	Water, reagent-grade			

Stable for 1 week at room temperature. ***Note:*** Or weigh 1.59 g of the pentahydrate.

640 mM Sodium thiosulfate



EPA Safer Choice low-concern (provisional)

☐ Flush to drain with excess water

Date: Sign: R0125

Sodium thiosulfate sensitizing solution

Amount	Ingredient		Stock	Final
250 µL	Sodium thiosulfate	R0125	640 mM	0.8 mM
To 200 mL	Water, reagent-grade			

Sodium thiosulfate sensitizing solution



EPA Safer Choice low-concern (provisional)

☐ Flush to drain with excess water

Date: Sign: R0126

Tetrathionate sensitizing solution

Amount	Ingredient		Stock	Final
10 g	Potassium acetate	[127-08-2]	98.15 g/mol	500 mM
605 mg	Potassium tetrathionate	[13932-13-3]	302.5 g/mol	10 mM
60 mL	Ethanol, non-denatured	[64-17-5]	100%	30%
	Flammable liquid (H225)			
To 200 mL	Water, reagent-grade			

Prepare the day of use. Stable for 1 day at room temperature.

Tetrathionate sensitizing solution

500 mM Potassium acetate, 10 mM Potassium tetrathionate, 30% Ethanol



DANGER

Flammable liquid

☐ Collect as ORGANIC SOLVENT WASTE

☐ Keep away from oxidizers

Date: Sign: R0127

Silver nitrate, 0.2%

Amount	Ingredient		Stock	Final
500 mg	Silver nitrate	[7761-88-8]	169.87 g/mol	0.2%
	Oxidizer (H272); Skin corrosion (H314)			
To 250 mL	Water, reagent-grade			

Store at room temperature, shielded from light.

0.2% Silver nitrate





Aquatic toxicity (long-term)

☐ Collect as HEAVY METAL WASTE

☐ DO NOT wash into sewer

Date: Sign: R0128

Silver nitrate developer

Amount	Ingredient		Stock	Final
30.4 g	Potassium carbonate	[584-08-7]	138.20 g/mol	220 mM
250 µL	Formaldehyde	[50-00-0]	37%	0.0093%
	Skin corrosion (H314); Acute respiratory toxicity (H330); Mutagenicity (H341); Carcinogenicity (H350); Organ toxicity, single exposure (H370, H371)			
80 µL	Sodium thiosulfate	R0125	640 mM	0.05 mM
To 1 L	Water, reagent-grade			

Prepare the day of use. Stable for 1 day at room temperature. ***Critical:*** Add formaldehyde no more than one hour before use to ensure consistent developer performance.

Silver nitrate developer

220 mM Potassium carbonate, 0.0093% Formaldehyde, 0.05 mM Sodium thiosulfate



DANGER

Carcinogenicity; Specific target organ toxicity; Skin sensitization; Germ cell mutagenicity

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0129

Tris stop solution

Amount	Ingredient		Stock	Final
40 g	Tris base	[77-86-1]	121.14 g/mol	4%
20 mL	Acetic acid, glacial	[64-19-7]	99.6%	2%
	Skin corrosion (H314)			
To 1 L	Water, reagent-grade			

Prepare the day of use. Stable for 1 day at room temperature.

[CLR06]

Tris stop solution

4% Tris base, 2% Acetic acid

No GHS hazards at the indicated concentrations

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0264

Potassium ferricyanide, 5%

Amount	Ingredient		Stock	Final
2.5 g	Potassium ferricyanide	[13746-66-2]	329.24 g/mol	5%
To 50 mL	Water, reagent-grade			

Note: This is a 150 mM stock solution.

5% Potassium ferricyanide

Aquatic toxicity (long-term)

- ☐ Collect as CYANIDE WASTE
- ☐ Keep alkaline, DO NOT acidify
- ☐ DO NOT wash into sewer

Date: Sign: R0130

Ammonium hydrogencarbonate, 200 mM

Amount	Ingredient	Stock	Final
1.58 g	Ammonium hydrogencarbonate [1066-33-7]	79.06 g/mol	200 mM
To 100 mL	Water, reagent-grade		

Prepare fresh before use.

200 mM Ammonium hydrogencarbonate

No GHS hazards at the indicated concentrations

- ☐ Hold for EHS review before disposal

Date: Sign: R0201

Silver nitrate destaining solution

Amount	Ingredient		Stock	Final
1.0 mL	Potassium ferricyanide	R0130	5%	15 mM
0.8 mL	Sodium thiosulfate	R0125	640 mM	50 mM
To 10 mL	Water, reagent-grade			

Prepare just before use. ***Critical:*** The yellowish solution is only active for 30 min; use immediately!

Silver nitrate destaining solution

15 mM Potassium ferricyanide, 50 mM Sodium thiosulfate

No GHS hazards at the indicated concentrations

- ☐ Collect as CYANIDE WASTE
- ☐ Keep alkaline, DO NOT acidify
- ☐ DO NOT wash into sewer

Date: Sign: R0171

List of references

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M. Chevallet, S. Luche, and T. Rabilloud, *Nat. Protoc.* **1**(4), 1852—1858 (2006).

F. Gharahdaghi, C. Weinberg, C. Weinberg, D. Meagher, B. Imai, and S. Mische, *Electrophoresis* **20**(3), 601—605 (1999).

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A. Shevchenko, M. Wilm, O. Vorm, and M. Mann, *Anal. Chem.* **68**(5), 850—858 (1996).

P. Sinha, J. Poland, M. Schnölzer, and T. Rabilloud, *Proteomics* **1**(7), 835-840 (2001).

Change log

2026-05-22 **T. Rabilloud, Benjamin C. Buchmuller** Version 1, 2006 to 2026. Initial, peer-reviewed protocol article, adapted as an SOP in 2023 and revised in 2025; the sensitizing solution and developer arithmetic reconciled in 2026 without changing the reagent chemistry.

2026-11-02 **Benjamin C. Buchmuller** Version 2. Sensitivity changes with the stain: thiosulfate as a 640 millimolar stock, the sensitizer at 0.8 mM, and new development and fixing times. Credits: Blum et al. (1987), Chevallet et al. (2006), Sinha et al. (2001), and the Invitrogen SilverQuest kit. Also corrected the developer formalin, the fixation, the silver nitrate hazard, the tetrathionate rinse and the stop solution.

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