In-gel protein staining with silver nitrate

Silver staining detects proteins after electrophoretic separation with high sensitivity down to 200 pg 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 (Chevallet et al., 2006), which uses formaldehyde-based image development in alkaline carbonate. Compared to other silver stains—such as silver-ammonia (Blum et al., 1987) 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 that reacts violently with combustible and reducing materials such as alkynes, alkalis, or halides**

▷ Wear gloves, safety glasses, lab coat

☐ Collect formaldehyde solutions as HAZARDOUS WASTE

☐ Collect silver containing solutions as SILVER WASTE

☐ Precipitate silver nitrate solutions immediately with sodium chloride or ascorbic acid to prevent formation of explosive silver azide

☐ DO NOT wash into sewer

Reviewed: Apr 5, 2025

Procedures

›› *Protein fixation*

☐ Fixation solution, 200 mL ⟨*R*⟩ ☐ 30% Ethanol, 400 mL

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

> ***Critical:*** Do not use aldehydes if mass spectrometry is planned. Fixation with methanol and acetic acid is sufficient but slightly less sensitive.

> ***Hint:*** Longer fixation (18 h) improves image quality. For mass spectrometry, fix for 20 min in a water bath slightly above room temperature to reduce crosslinking. Keep in mind that this increases background and may impair quantification.

> ***Hint:*** Use at least 4- to 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. 40 min

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

›› *Sensitization and impregnation with silver nitrate*

- ☐ Sodium thiosulfate sensitizing solution, 200 mL ⟨*R*⟩
- ☐ Tetrathionate sensitizing solution, 200 mL ⟨*R*⟩
- ☐ 0.2% Silver nitrate, 250 mL ⟨*R*⟩

(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:

- 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. 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, 200 mL ⟨*R*⟩
- ☐ 2% Acetic acid, 200 mL

(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 2% acetic acid. 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

(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

- ☐ 5% Potassium ferricyanide, 50 mL ⟨*R*⟩
- ☐ Silver nitrate destaining solution, 10 mL ⟨*R*⟩

(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.

Recipes

Fixation solution

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

Fixation solution

30% Ethanol, 10% Acetic acid

WARNING

Flammable liquid; Eye irritation; Skin irritation

☐ Collect as HAZARDOUS WASTE

Date: Sign: R0170

Sodium thiosulfate, 10%

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

Stable for 2 weeks at 4 °C. ***Note:*** This is a 630 mM stock.

10% Sodium thiosulfate

Skin irritation

Expiry: Sign: R0125

Sodium thiosulfate sensitizing solution

Amount	Ingredient		Stock	Final
250 µL	Sodium thiosulfate	R0125	10%	0.01%
To 200 mL	Water, reagent-grade			

Sodium thiosulfate sensitizing solution

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%
To 200 mL	Water, reagent-grade			

Prepare the day of use. Stable for 1 day.

Tetrathionate sensitizing solution

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

DANGER

Reproductive toxicology; Flammable liquid; Serious eye irritation; Skin irritation

☐ Collect as HAZARDOUS WASTE

Expiry: Sign: R0127

Silver nitrate, 0.2%

Amount	Ingredient		Stock	Final
50 mg	Silver nitrate	[7761-88-8]	169.87 g/mol	0.2%
To 250 mL	Water, reagent-grade			

Store at 4 °C.

0.2% Silver nitrate

WARNING

Hazardous to the aquatic environment

☐ Collect as SILVER WASTE

☐ DO NOT empty into drains

Date: Sign: R0128

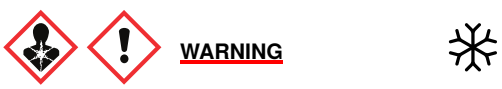
Silver nitrate developer

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%	0.01%
125 µL	Sodium thiosulfate	R0125	10%	0.01%
To 1 L	Water, reagent-grade			

Prepare the day of use. Stable for 1 day at 4 °C.

Silver nitrate developer

220 mM Potassium carbonate, 0.01% Formaldehyde, 0.01% Sodium thiosulfate



WARNING

Carcinogenicity; Serious eye irritation; Skin irritation

☐ Collect as HAZARDOUS WASTE

Expiry: Sign: R0129

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

WARNING

Hazardous to the aquatic environment; Serious eye irritation

☐ DO NOT empty into drains

Date: Sign: R0130

Silver nitrate destaining solution

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

Prepare just before use.

Silver nitrate destaining solution

15 mM Potassium ferricyanide, 50 mM Sodium thiosulfate

Date: Sign: R0171

List of references

H. Blum, H. Beier, and H.J. Gross, *Electrophoresis* **8**(2), 93-99 (1987).

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

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

Change log

2006-11-22	T. Rabilloud	Initial, peer-reviewed protocol article.
2023-01-16	Benjamin C. Buchmuller	Adaptation as SOP.
2025-04-05	Benjamin C. Buchmuller	Revised protocol sections.