In-gel protein staining with silver nitrate

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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.

This is a bench card. Full protocol available online.

Procedures

›› ***Protein fixation***

- ☐ R0170 Fixation solution, 200 mL
- ☐ 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.

(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

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›› *Sensitization and impregnation with silver nitrate*

- ☐ R0126 Sodium thiosulfate sensitizing solution, 200 mL
- ☐ R0127 Tetrathionate sensitizing solution, 200 mL
- ☐ R0128 0.2% Silver nitrate, 250 mL

(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.

›› *Image development*

- ☐ R0129 Silver nitrate developer, 200 mL
- ☐ 2% Acetic acid, 200 mL

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

(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.

(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.

Destaining for mass spectrometry

- ☐ R0130 5% Potassium ferricyanide, 50 mL
- ☐ R0171 Silver nitrate destaining solution, 10 mL

(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. 🕮

List of references

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

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