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Sodium thiosulfate-free silver staining for PAGE

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We are still developing and optimizing this protocol

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Abstract

This protocol describes a silver staining method for visualizing proteins separated by SDS-PAGE without using sodium thiosulfate as a sensitizer. The procedure involves fixation of the gel to immobilize proteins and reduce background, followed by impregnation with a silver nitrate solution that binds to the protein bands. After rinsing off excess silver, the gel is developed using a sodium carbonate and formaldehyde solution, which reduces silver ions to metallic silver at protein locations, creating visible bands. The reaction is stopped with a Tris-acetic acid solution to stabilize the image and prevent overdevelopment. Final washes remove residual chemicals, resulting in sensitive protein detection with minimal background. This thiosulfate-free protocol is recommended when downstream applications like mass spectrometry require avoidance of certain chemical interferences.

Guidelines

- **Handle solutions with care:** Prepare silver nitrate and developing solutions fresh or store properly in dark containers to prevent degradation.
- **Protect from light:** Perform silver impregnation and development steps in low light or darkness to avoid nonspecific silver reduction and background staining.
- **Optimize fixation:** Ensure thorough fixation of the gel to reduce background and enhance protein band clarity.
- **Wash thoroughly:** Adequate washing between steps is critical to remove excess reagents and minimize background staining.
- **Monitor development time:** Observe the gel carefully during development; stop the reaction promptly when bands are visible to prevent overdevelopment and high background.
- **Be aware of reduced sensitivity:** Omitting sodium thiosulfate may reduce staining sensitivity and contrast; adjust staining times accordingly.
- **Use appropriate downstream processing:** This protocol is particularly useful when avoiding thiosulfate is necessary, such as prior to mass spectrometry analysis.
- **Dispose of waste properly:** Silver-containing solutions are hazardous; follow institutional guidelines for disposal.

Materials

Fixing Solution (500 mL)
- Ethanol (100%): 250 mL
- Glacial acetic acid: 50 mL
- Ultrapure water: 200 mL

Mix thoroughly and store at room temperature.

Silver Nitrate Solution (0.1-0.2%)
- Silver nitrate (AgNO3): 1-2 g
- Ultrapure water: 1 L

Prepare carefully and store in a dark bottle protected from light.

Developing Solution
- Sodium carbonate (Na2CO3) 2% (w/v): 20 g dissolved in 1 L ultrapure water
- Formaldehyde (37%): Add 0.5 mL per 100 mL of sodium carbonate solution (final 0.05%)

Prepare fresh before use.

Stopping Solution
- Tris base 50 mM: Dissolve 6.06 g in 1 L ultrapure water
- Acetic acid 10 mM: Add 0.6 mL glacial acetic acid per 1 L Tris solution

Adjust pH to ~7.0

Safety warnings

- **Omission of Thiosulfate Sensitization:** This protocol does not include a sensitization step with sodium thiosulfate to avoid chemical interferences that can affect downstream analyses such as mass spectrometry. Thiosulfate can form complexes with silver ions, which may cause protein modifications or artifacts during staining.
- **Reduced Sensitivity and Contrast:** Without sodium thiosulfate, the sensitivity of protein detection may be lower, and background staining can be higher. Careful optimization of fixation, washing, and development times is critical to obtain clear and specific protein bands.
- **Handling Silver Solutions:** Silver nitrate and related reagents are light-sensitive and should be protected from light to prevent nonspecific silver reduction. Silver-containing waste is hazardous; dispose of it according to institutional regulations and environmental safety guidelines.
- **Use of Chemicals:** Formaldehyde used in development is toxic and volatile; handle it in a fume hood using appropriate personal protective equipment (PPE). Acetic acid and ethanol can irritate the skin and eyes; wear gloves and eye protection.
- **Monitoring Development Time:** Overdevelopment can lead to dark background and mask protein bands. Development should be stopped promptly once clear bands appear.
- **Gel Handling:** Avoid contamination of gels with metallic objects or dirty containers, which can cause precipitation of silver and artifacts.
- **Temperature Control:** Staining reactions are temperature sensitive. Perform silver impregnation and development at recommended conditions to ensure reproducibility.

Fixation

1h 30m

1 Immerse gel in Fixing Solution for 00:30:00 at Room temperature with gentle agitation.

30m

2 (Optional) It can be also fixed for 01:00:00 at Room temperature or Overnight at 4 °C to reduce background.

1h

Washing

10m

3 Wash gel 3 times for 00:10:00 each in ultrapure water with gentle agitation.

10m

Silver Impregnation

30m

4 Incubate gel in Silver Nitrate Solution [M] 0.1-0.2 Mass Percent for 00:30:00 at Room temperature in the dark or low light.

30m

Quick Washes

30s

5 Rinse gel twice in ultrapure water for 00:00:30 to remove excess silver nitrate.

30s

Development

10m

6 Prepare fresh Developing Solution.

7 Immerse gel in Developing Solution and monitor band development for a maximum of 00:10:00 .

10m

8 Stop development when bands are visible and before background darkens.

Stopping Development

5m

9 Transfer gel to Stopping Solution for 00:05:00 to stabilize staining. 5m

Final Wash

15m

10 Wash gel in ultrapure water for at least 00:15:00 to remove residual chemicals. 15m

Storage and Documentation

11 Store gel in ultrapure water or document bands by photographing.

Protocol references

Chevallet, M., Luche, S., & Rabilloud, T. (2006). Silver staining of proteins in polyacrylamide gels. *Nature protocols*, *1*(4), 1852–1858. https://doi.org/10.1038/nprot.2006.288.

Gharib, M., Marcantonio, M., Lehmann, S. G., Courcelles, M., Meloche, S., Verreault, A., & Thibault, P. (2009). Artifactual sulfation of silver-stained proteins: implications for the assignment of phosphorylation and sulfation sites. *Molecular & cellular proteomics : MCP*, *8*(3), 506–518. https://doi.org/10.1074/mcp.M800327-MCP200.

Waggoner, B., Newman, T., & Tarbutton, T. (2016). Quick, safe, and simple silver staining for ciliates and other protists. *Tested Studies for Laboratory Teaching*, 37, Article 63. Proceedings of the 37th Conference of the Association for Biology Laboratory Education (ABLE).