Protocol for Silver Staining of Gels

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GUIDELINES

Silver staining is used for sensitive detection of proteins separated by 1D and 2D SDS PAGE with detection limits from 0.5-5 ng. Many silver staining protocols and commercial staining kits are not compatible with mass spectrometry due to the use of cross-linking reagents. Commercial silver staining kits compatible with mass spectrometry are available from different suppliers, including ProteoSilver Plus (Sigma) and Dodeca Silver Stain (BioRad).

Here we describe a silver staining protocol that has been optimized for mass spectrometric analysis. The protocol results in confident protein identifications and high sequence coverage by MALDI MS and ESI MS due to a high recovery of peptides from the stained gel. The protocol has been tested and documented in many publications (Mortz, E et al, Proteomics 1 (11), 1359-63, 2001).

MATERIALS

Use high quality chemicals and ultra pure water (18.2 Mohm). Agitate the gels and perform work in a fume hood. Wear gloves at all stages and use clean staining trays with lids to avoid keratin contamination of the gels.

PROCEDURE

1. Run 1D or 2D gel. Incubate the gel in Fixer (40% ethanol, 10% acetic acid, 50% H_2O) for 1 hr.
2. Wash the gel in H_2O for at least 30 min. NB! Overnight washing with several changes of water will remove all acetic acid, reduce background staining and increase sensitivity.
3. Sensitize the gel in 0.02% sodium thiosulfate (0.04 g $Na_2S_2O_3$, 200 ml H_2O) for only 1 min. NB! Longer time will decrease peptide recovery from the gel.
4. Wash gel in H_2O for 3 x 20 sec.
5. Incubate gel for 20 min in 4°C cold 0.1% silver nitrate solution (0.2 g $AgNO_3$, 200 ml H_2O, 0.02% formaldehyde (add 40 µL 35% formaldehyde just before use). NB! Staining is enhanced with cold $AgNO_3$.
6. Wash the gel in H_2O for 3 x 20 sec.
7. Place the gel in a new staining tray. NB! Residual $AgNO_3$ on the gel surface and staining tray will increase background staining.
8. Wash the gel in H_2O for 1 min.
9. Develop the gel in 3% sodium carbonate (7.5 g Na_2CO_3 in 250 ml H_2O), 0.05% formaldehyde (add 125 µL 35% formaldehyde just before use). NB! Change developer solution immediately when it turns yellow. Terminate when the staining is sufficient.
10. Wash the gel in H_2O for 20 sec.
11. Terminate staining in 5% acetic acid for 5 min.
12. Leave the gel at 4 °C in 1% acetic for storage. Prior to MS analysis the gel is washed in water for 3 x 10 min to ensure complete removal of acetic acid.

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