Silver Staining Method (MS compatible)

Reagents (*prepare all solutions fresh immediately prior to use*)

Fixing solution (50% methanol, 10% glacial acetic acid, 0.05% formalin)
Wash solution (35% ethanol)
Sensitization solution (0.02% $Na_2S_2O_3$)
Staining solution (0.2% $AgNO_3$, 0.076% formalin)
Developing solution (6% Na_2CO_3, 0.05% formalin, 0.0004% $Na_2S_2O_3$)
Stop solution (50% methanol, 10% glacial acetic acid)

Procedure

1. Fix gel in Fixing solution for 2 hr to overnight with gentle agitation.
2. Wash gel in Wash solution for 20 min. Repeat 2 more times.
3. Sensitize gel in Sensitization solution for 2 min.
4. Wash gel in ddH_2O for 5 min. Repeat 2 more times.
5. Stain gel in Staining solution for 20 min with gentle agitation.
6. Wash gel in ddH_2O for 1 min. Repeat 1 more time.
7. Develop gel in Developing solution until the staining is sufficient.
8. Stop staining by soaking the gel in Stop solution for 5 min. Change the solution a couple of times.
9. Wash gel in ddH_2O for 5 min. Repeat 2 more times. The gel is now ready for scanning and spot removal for MS.
10. Store the gel at 4^{o}C in 1% acetic acid.

Note

1. *Prepare all solutions in clean glassware using ddH_2O or milliQ H_2O with a resistivity >16 mho/cm.*
2. *Use ~250 ml of each solution per gel (18 x 20 x 0.1 cm).*
3. *Handle gel with powder-free gloves.*
4. *Staining trays should be very clean.*
5. *The gel should be scanned as soon as possible.*

Reference:

Mortz, E., Krogh, T. N., Vorum, H., and Görg, A. (2001) Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis, 1: 1359-1363.