Silver Stain Protocol

1. Fix gel in 40% EtOH, 10% acetic acid for 30 min.
2. Wash gel in 30% EtOH for 20 min, repeat once.
3. Wash gel in H_2O for 20 min.
4. Sensitize gel in 0.02% $Na_2S_2O_3$ for 1 min.
5. Wash gel in H_2O for 20 sec, repeat twice.
6. Incubate gel in cold 0.1% $AgNO_3$ for 20 min at 4^oC.
7. Wash gel in H_2O for 20 sec, repeat twice.
8. Develop gel in 3% Na_2CO_3 , 0.05% formalin

   <Note> Observe the color and change solution when the developer turns yellow. Terminate when the staining is sufficient.
9. Terminate staining in 5% Acetic acid.
10. Wash the gel in H_2O for 10 min, repeat twice.
11. Leave the gel at 4^oC in 1% Acetic acid.

Reference: Mortz, E *et al*. Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis. *Proteomics*. 2001, 1, 1359-1363