High-Sensitivity Silver Staining of SDS-PAGE Gels

Reagents

50% MeOH, 12% acetic acid, and 100 μL 37% paraformaldehyde solution per 200 mL (Fix Solution)
50% MeOH (Wash Solution)
0.02% (w/v) $Na_2S_2O_3$ (Pretreatment Solution)
0.2% (w/v) $AgNO_3$ (Silver Solution)
6% Na_2CO_3 and 4 mL 0.02% $Na_2S_2O_3$/100 μL 37% paraformaldehyde solution per 200 mL (Develop Solution)
50% MeOH, 12 % acetic acid (Stop Solution)
30% MeOH, 5% glycerol (Drying Solution)

Procedure

1) Treat gel with the Fix Solution for at least 1 hour (can be done overnight). 200 mL of Fix Solution is enough for 2-4 minigels.
2) Wash the gel with the Wash Solution for 3 x 8 minutes.
3) Treat the gel with the Pretreatment Solution for ***exactly*** 1 minute. The gel will float at first, and so care should be taken to uniformly treat the gel by agitation.
4) Rinse the gel with MilliQ water for ***exactly*** 3 x 20 seconds.
5) Incubate the gel with the Silver Solution for 20 minutes
6) Rinse the gel with MilliQ water for ***exactly*** 2 x 30 seconds.
7) Incubate the gel with the Develop Solution with agitation until the desired level of staining is achieved (typically 2-10 minutes). Be sure to have the Stop Solution ready.
8) Stop the staining by treating the gel with the Stop Solution for at least 10 minutes.
9) If you want to preserve the gel between sheets of porous plastic, wash the gel with the Wash Solution for 2 x 10 minutes. Then incubate the gel with the Drying Solution for a maximum of 1 hour (the glycerol will begin to leach out the silver after 1-2 hours).

Notes

1) This procedure can reliably detect protein levels down to about 5 ng.
2) All steps should be conducted with mechanical or manual swirling.
3) For more control over the staining rate, the Develop Solution can be removed from the gel when about 80% of the desired intensity is achieved. The gel will continue to develop slowly with the remaining reagents trapped in the gel.