

Silver staining protocol

Compatible with mass spectrometry, excellent signal-to-noise ratio

(Ref: Protana website <http://www.protana.com/services/protocols/default.asp>)

Buffers

Fixer: 50% MeOH, 12% HAc, 0.05% formalin (35% Formaldehyde) 200 ml

100 ml MeOH (99.8%)

24 ml HAc (100%)

100 µl formalin (35% Formaldehyd)

76 ml H₂O (Milli-Q)

Wash: 35% EtOH (96%) 200 ml

73 ml EtOH

127 ml H₂O

Sensitizing: 0.02% Na₂S₂O₃ 200 ml

0.04 g Na₂S₂O₃

200 ml H₂O

Silver nitrate:0.2% AgNO₃, 0.076% formalin (35% Formaldehyde) 200 ml

0.4 g AgNO₃

152 µl formalin (35% Formaldehyde)

200 ml H₂O

**Developer: 6% Na₂CO₃, 0.05% formalin (35% Formaldehyde),
0.0004% Na₂S₂O₃ 400 ml**

24 g Na₂CO₃

200 µl formalin (35% Formaldehyde)

8 ml 0.02% Na₂S₂O₃

392 ml H₂O

Stop solution:50% MeOH, 12% HAc 200 ml

100 ml MeOH

24 ml HAc
76 ml H₂O

Procedure

1. Fix gel: 50% MeOH, 12% HAc, 0.05% formalin, 2 hrs or overnight
2. Wash gel: 35% EtOH for 20 mins.
3. Wash gel: 35% EtOH for 20 mins.
4. Wash gel: 35% EtOH for 20 mins.
5. Sensitize gel: 0.02% Na₂S₂O₃ for 2 mins.
6. Wash gel: H₂O for 5 mins.
7. Wash gel: H₂O for 5 mins.
8. Wash gel: H₂O for 5 mins.
9. Stain gel: 0.2% AgNO₃, 0.076% formalin for 20 mins.
10. Wash gel: H₂O for 1 min.
11. Wash gel: H₂O for 1 min.
12. Develop gel: 6% Na₂CO₃, 0.05% formalin, 0.0004% Na₂S₂O₃
13. Stop staining: 50% MeOH, 12% HAc for 5 mins.
14. Leave the gel at 4°C in: 1% HAc

Remark: For coomassie blue stained gel, wash O/N in water, then start from step 5.