

SILVER STAINING

Guidelines for sample preparation
(How to protect your samples from contamination with keratin)

- Clean your bench
 - Try to avoid contact of samples and solutions with dust, skin or hair
 - Wear gloves at all times
 - All reagents should be prepared fresh
 - Use ultra-pure water for all solutions
1. Fix the gel for 1 h in fixing solution (40 % EtOH, 10 % Acetic Acid in MilliQ-H₂O)
 2. Wash in 30 % EtOH 2x 20 min
 3. Wash in MilliQ-H₂O for 20 min
 4. Wash gel in 0.02 % Na₂S₂O₃ for 1 min
0.1 g Na₂S₂O₃ in 500 ml H₂O (this solution must be freshly prepared every time)
 5. Wash gel in MilliQ-H₂O 2 x 20 seconds
 6. Incubate the gel in cold 0.1 % AgNO₃ solution for 20 min at 4 °C
0.5 g in 500 ml
 7. Wash in H₂O three times for 20 s
 8. Transfer the gel into a fresh clean tray
 9. Wash in H₂O for 1 min
 10. Develop the gel in 3 % Na₂CO₃, 0.05 % formalin solution (fresh!!).
Change developing solution when it starts to turn light brown.
Develop until bands are visible. Do not over-stain!
15 g Na₂CO₃
250 µl formalin
500 ml MilliQ-H₂O
 11. Wash the gel in MilliQ-H₂O for 20 s
 12. Fix the gel in 5 % acetic acid for at least 5 min
 13. Wash the gel at least once in MilliQ-H₂O
 14. For long-term storage, keep the gel at 4 °C in 1 % acetic acid (in MilliQ-H₂O) solution