

Silver stain modified acc. to Blum et al.

Solutions:

Fix :	40% EtOH, 10% HAc (400 ml EtOH 100 ml HAc ad 1000 ml with H ₂ O)
Wash:	30% EtOH (300 ml EtOH ad 1000 ml with H ₂ O)
Thiosulfate Solution: (Sodium Thiosulfate)	0.02% Na ₂ S ₂ O ₃ (20 mg Na ₂ S ₂ O ₃ in 100 ml H ₂ O)
Silver Nitrate Solution:	0.2% AgNO ₃ (200 mg AgNO ₃ in 100ml H ₂ O)
Developing Solution:	3% Na ₂ CO ₃ , 0.05% H ₂ CO, 0.0004% Na ₂ S ₂ O ₃ (3 g Na ₂ CO ₃ , 50 µl H ₂ CO (37%), 0.4 mg Na ₂ S ₂ O ₃ in 100 ml H ₂ O)
Stop solution:	0.5 % Glycine (5 g Glycine in 1000 ml)

Procedure:

Fix:	2hrs or O/N
Wash:	3x20 min
Thiosulfate:	1 min
Water wash:	3x 20 sec
Silver nitrate:	1 hr
Water wash:	3x 20 sec
Develop:	5-10 min
Water wash:	1 min
Stop:	5 min
Water:	30 min

!!!!ALWAYS USE GLOVES AND A CLEAN STAINING TRAY!!!!

!!!AVOID TOUCHING THE GEL!!!

For MALDI-TOF Analysis the cut bands should be destained according to the following protocol:

Destainig of silver stained bands

1. Silver stained gels are usually stored in 1% acetic acid at 4 degrees C. The residual acetic acid should be removed by thoroughly rinsing the gel with water before destaining.

2. The reducers, potassium ferricyanide (30mM) and sodium thiosulfate (100mM), should be made fresh. Mix the two reducers in a 1:1 ratio and immediately add the reducers to cover the gel pieces. Once the silver brown color disappears, remove the reducers and wash with water until the gel piece is clear (Note: Incubation after washing with water in ammonium bicarbonate (100mM) will speed this process). Make sure that the gel piece is clear before proceeding with digestion.

**!!!!PLEASE AVOID MULTIPLE TRANSFERS OF THE BANDS BETWEEN
DIFFERENT TUBES !!!!**