

We use this silver staining procedure exclusively in the laboratory. It is relatively rapid, as sensitive (~1 ng) as all other silver stains, and is reversible (see below). We recommend to not stray from suggested times as it will introduce some variability to your results.

SOLUTIONS

Fixative: Acetic acid 10% (v/v), ethanol 30% (v/v)

*Measure 100 ml Glacial Acetic Acid
Measure 300 ml ethanol
To 1000 ml with MilliQ water*

Rinse: 20% (v/v) ethanol

Sensitizer: 0.02% (w/v) sodium thiosulfate

*Weigh 0.2 gm sodium thiosulfate
Dissolve in 1000 ml MilliQ water*

Silver nitrate: 0.2% (w/v) silver nitrate

*Measure 2.0 gm silver nitrate
Dissolve in 1000 ml MilliQ water*

Developer: 0.3% (w/v) sodium carbonate
0.025% (v/v) formaldehyde
sodium thiosulfate 10 mgm/L

*Measure 15 gm sodium carbonate
Measure 25 ml of stock sodium thiosulfate
Measure 125 ul 37% formaldehyde
To 500 ml with MilliQ water*

Stop: Tris, acetic acid

*Measure 50 gm Tris base
Measure 25 ml Glacial acetic acid
To 1000 ml with MilliQ water*

PROCEDURE

1. Soak the gel in Fixative for at least one hour; change solution for a minimum of another hour; overnight is ok.
2. Rinse gel in Rinse Solution for 20 min.
3. Rinse gel in MilliQ water for 10 min.
4. Soak gel in Sensitizer Solution for 1 min.
5. Rinse gel in MilliQ water for 3 x 20 seconds.
6. Soak gel in Silver Nitrate Solution for 45 min.
7. Rinse gel with MilliQ water 5-10 seconds.
8. Soak gel in Developer Solution until bands are adequate.
9. Soak gel in Stop Solution for minimum 5 minutes.

DESTAINING SILVER STAINED GEL SLICES (for Blum silver stained gels)

Solutions needed: (PREPARE FRESH)

30 mM potassium ferricyanide; 98 mg/10 ml water
100 mM sodium thiosulfate; 248 mg/10 ml water

Procedure:

mix solutions together 1:1 (this is working solution)
Pipet 50 μ l of working solution onto gel slice(s)
Incubate until slice is destained (several minutes); stop with water; discard
Cover with 250 μ l of 100mM ammonium bicarb, 20 min; discard