SOP FOR NON-FIXING SILVER STAIN FOR SDS POLYACRYLAMIDE GELS (BLUM METHOD)

(Ref: Blum, H., Beier, H. and Gross, H.J. (1986). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis 8: 93-99.)

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: **MUST BE PREPARED FRESH**

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 (minigel) or 20 min (13 x 16cm gel).

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. **(MAKE DEVELOPER NOW)**

7. Rinse gel with MilliQ water 5-10 seconds. THIS IS A CRITICAL TIME. IT MEANS **SECONDS, NOT MINUTES.**

8. Soak gel in Developer Solution until bands are adequate (typically 8-10 minutes)

9. Soak gel in Stop Solution for minimum 15 minutes, then keep in water.

THE GEL STAINING PROCESS IS COMPLETE. IT DOES NOT REQUIRE DESTAINING LIKE A COOMASSIE STAINED GEL. DESTAIN ONLY SLICES THAT WILL BE DIGESTED.

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

Solutions needed: (make 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 μl of working solution onto gel slice(s)
Incubate until slice is destained (several minutes); stop with water; discard
Cover with 250 μl of 100mM ammonium bicarb, 20 min; discard