

SILVER GEL

Wash plates in HNO₃/distilled H₂O, 1:1, 30 min., rinse with H₂O, dry.

Pour gel using reagents containing distilled H₂O

Run gel in running buffer made with distilled H₂O

Tris	2.27 g (tris base)
Glycine	10.80
SDS	0.75
Distilled H ₂ O	<u> </u>
	750 ml

Pre-fix in 50% MeOH, 30 min.
10% glacial acetic acid

Wash in 5% MeOH, 7% acetic acid ~2 hr or overnight

Wash 30 min in H₂O

Fix in 10% glutaraldehyde, 30 min. Dilute 50% stock 20 ml to 100 ml
(glutaraldehyde is available from Kodak)

Wash extensively with H₂O ~4 hr or overnight (repeated changes)

Add silver stain – 15 min

- (a) Mix 20.4 ml H₂O with 0.63 ml 3N NaOH
- (b) Add fresh NH₄OH* 1.4 ml
- (c) Add 4 ml AgNO₃ with swirling
(Dissolve 0.8 g AgNO₃ into 4 ml H₂O)
- (d) Dilute to 100 ml with H₂O

Wash gel in new dish with H₂O (lots) 2 min. Change H₂O 2 X. Shake gently.

Place gel in new dish with developer

1% citric acid	2 ml
39% formaldehyde	200 λ
Distilled H ₂ O	<u> </u>
	400 ml

Stop development with H₂O

Bleach appropriately with fixer. (Kodak Rapid Fixer Solution A, diluted 1:4)

Stop bleaching action with Hypo (Kodak Hypo clearing solution, diluted 1:4)

Sensitivity – 5 ng is clearly visible

*Fresh NH₄OH means one that has been kept tightly capped and less than 1 year old