

Note:

The following research protocol is intended for use only by qualified personnel in a properly equipped laboratory and who have been trained in the safe handling of chemicals.

Please dispose of all chemicals safely and in accordance with applicable regulations.

Silver Stain for Polyacrylamide Gels¹

Reagents:

- Fixer: 50% ethanol, 5% acetic acid, in water
- Wash: 50% ethanol in water
- Sensitizer: 0.02% sodium thiosulfate
- Stain: 0.1% silver nitrate, in water
- Developer: 0.04% formalin in 2% sodium carbonate
- Stop: 5% acetic acid in water
- Storage: 1% acetic acid in water

Procedure:

- a) 30 minutes in Fixer (can be left longer)
- b) 10 minutes in Wash
- c) 2 × 10 minutes in water
- d) 2 minutes in Sensitizer
- e) 2 × 3 minutes in water
- f) 30 minutes in Stain
- g) 1 minute in water
- h) add a small amount of Developer, swirl briefly and discard
- i) add more Developer and shake slowly until spots/bands appear
- j) 5 minutes in Stop solution
- k) store in Storage solution at 4°

Destain Procedure²

(Optional: if staining gave poor results, you can destain and try again)

Reagents:

- Solution A: 30 mM potassium ferricyanide
- Solution B: 100 mM sodium thiosulfate
- Destain: mix solution A and solution B in a 1:1 ratio just before use

Method:

- a) Immerse the gel in Destain
- b) Brownish colour should be removed within 3-5 minutes
- c) Wash 3 × 10 minutes with deionized water or until yellow colour is removed

¹Based upon Shevchenko *et al.* Analytical Chemistry (1996) 68:850-858.

²Based upon Gharahdaghi *et al.* Electrophoresis (1999) 20:601-605.