

SILVER STAINING

FIXER:

20 mL dH₂O
25 mL methanol
5 mL acetic acid

DEVELOPER:

1.5 g Na₂CO₃
125 uL formaldehyde
250 mL dH₂O

PROTOCOL:

Put (0.75 mm) gel into fixer and rotate 15 minutes.

Wash 3x with dH₂O, 5 minutes each wash.

Add 50 mL dH₂O with 3.5 uL of 1M DTT and rotate 10 minutes.

Add 50 mg AgNO₃ and 50 mL dH₂O and rotate 10 minutes.

Add developer in about 5 portions. The first portion should turn cloudy/grey suddenly. When this occurs, pour it off and replace with more developer. Wait until either the developer appears to be cloudy or no changes in staining occur before adding the remaining 3 portions of developer.

Stop in 5% acetic acid for 5-10 minutes.

Put gel into 3% glycerol. Wet gel drying paper in 3% glycerol and lay flat on gel dryer.

Place gel on paper and fold over the rest of it, trapping as few bubbles as possible.

Dry at 70-80°C for 1-2 hours.

For a 1.5 mm gel, all incubations should be twice as long.

