

## Ammoniacal Silver Staining of Electrophoretic gels

### Solutions:

#### 1. Ethanol fixative

	200 ml
50% Ethanol	100 ml ethanol absolute
10 % Acetic acid	20 ml
Water	100 ml

#### 2. Glutaraldehyde fixative

	150 ml
Glutaraldehyde (25 %)	60 ml
Water	90 ml

#### 3. Silver-nitrate stock

	100 ml
1.14 M AgNO <sub>3</sub>	19.365 g

#### 4. NaOH stock

	500 ml
90 mM NaOH	1.8 g

#### 5. Citric acid stock

	250 ml
47.6 mM Citric acid	2.5 g

#### 6. Ammoniacal silver stain solution (prepared fresh)

	150 ml
Water	110.0 ml
NH <sub>4</sub> OH	1.75 ml (concentrated)
NaOH	31.5 ml stock
AgNO <sub>3</sub>	6.0 ml stock

\*Note: Mix ingredients, place beaker on stirrer. Then, slowly add extra silver nitrate, drop-by-drop, until the solution becomes saturated, and turns light yellow-brown.

#### 7. Developer

	400 ml
Citric acid	2 ml stock
Formaldehyde	0.2 ml
Water	397.8 ml

#### 8. Also needed:

- a. lots of distilled water
- b. preparations for photography: Kodak TechPan, 50ASA, 1/30s

**Steps:**

1. Fix gel with Ethanol fixative for 45 minutes.
2. Fix gel with Glutaraldehyde fixative overnight.
3. Wash gel with water. Several changes of water until glutaraldehyde is completely washed out. Often a whole day of washing is necessary.
4. Silver stain for 5 minutes. After preparing the Silver stain solution fresh (!!), add the whole amount (150 ml) on the gel.
5. Wash gel with ~200 ml water.
  - a. 1 minute
  - b. 3 minutes
6. Develop gel until bands appear. Avoid overdeveloping the gel. Best is to slightly underdevelop, because the developing procedure will be continuing after stopping the development.

Add 200 ml of developer on gel.  
May repeat the addition of developer after 5 minutes.
7. Stop development reaction by washing gel with profuse amounts of water.
8. Photograph gel on light box. Use 1/30 exposure setting and a range of 2.8-32 f-stop settings.
9. Continue washing of gel with water for about an hour.
10. Wrap gel-box in aluminum foil to protect gel from light.