

## Silver Staining

Nabila Aboulaich

### **Materials and Reagents**

1. 99.5 % ethanol (Sigma-Aldrich)
2. Silver nitrate ( $\text{AgNO}_3$ ) (Sigma-Aldrich)
3. Formaldehyde solution (37 %) (Sigma-Aldrich)
4. Sodium carbonate (Sigma-Aldrich)
5. Sodium thiosulphate (Sigma-Aldrich)
6. EDTA (Sigma-Aldrich)
7. Sodium dithionite (Sigma-Aldrich)
8. 30% ethanol
9. Acetic acid
10. Stop solution (see Recipes)
11. Fixation solution (see Recipes)
12. Sensitizer solution (see Recipes)
13. Staining solution (see Recipes)
14. Developer solution (see Recipes)

### **Equipment**

1. Shaker

### **Procedure**

1. Add fixation solution on gel and incubate overnight or minimum 2 h with gentle shaking.
2. Wash gel with gentle shaking with 30% ethanol 3x 10 min.
3. Wash gel with dest. water 2x 10 min.
4. Incubate gel with sensitizer solution for 1 min.
5. Wash gel with dest. water 2x 1min.
6. Incubate gel with staining solution for 25 min.
7. Wash gel with dest. water for 1 min.
8. Add developer solution (about 2-3 min) with gentle mixing.
9. Add stop solution before the protein bands get too dark stained.

## Recipes

### 1. Fixation solution

30% ethanol      160 ml

10% acetic acid   50 ml

H<sub>2</sub>O                290 ml

### 2. Sensitizer solution

Sodium dithionite 25 mg/100 ml (sensitizer, always make it fresh)

### 3. Staining solution

0.2% AgNO<sub>3</sub> (400 µl/40 ml from 20% AgNO<sub>3</sub> stock solution from the freezer)

Careful, it is strong oxidizer and blackens everything)

+ 3 µl/40 ml formaldehyde solution (36-38%, must be fresh)

### 4. Developer solution

6% sodium carbonate (3 g/50 ml)

4 µg/ml sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 50 µl of stock solution 4 mg/ml, can be stored a few weeks in the fridge)

+ formaldehyde 25 µl/50 ml. Always make developer fresh

*Note: Do not use more formaldehyde than in therecipe, it might produce background. For better result, use fresh stock solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (4 mg/ml).*