Silver Staining

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Materials and Reagents

1. 99.5 % ethanol (Sigma-Aldrich)
2. Silver nitrate ($AgNO_3$) (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_2O 290 ml
2. Sensitizer solution
   Sodium dithionite 25 mg/100 ml (sensitizer, always make it fresh)
3. Staining solution
   0.2% $AgNO_3$ (400 µl/40 ml from 20% $AgNO_3$ 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 ($Na_2S_2O_3$, 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 then in therecipe, it might produce background. For better result, use fresh stock solution of $Na_2S_2O_3$ (4 mg/ml).