Silver Staining of Protein Gels

Reference

Morrissey, J.H. (1981). Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117**: 307-310.

Reagents

Silver Solution:

0.1% $AgNO_3$ in H_2O: 0.1 g $AgNO_3$ dissolved in 100 ml H_2O.

Developing Solution:

3% sodium carbonate (Na_2CO_3) in H_2O

Add 50 µl formaldehyde/100 ml, and stir for 2 min.

Procedure

All incubations are at room temperature, and carried out with gentle shaking on a shaking platform.

All steps take place in a clean (acid-washed) Pyrex dish.

Make up all solutions fresh, just before use.

1. Incubate 15 min in 50% MetOH.
2. Incubate 15 min in 5% MetOH.
3. Incubate 15 min in 32 µM DTT (8 µl 1M DTT /250 ml water) solution (freshly made).
4. Wash briefly (approx 5 to 10 sec) with H_2O, twice.
5. Wash with a little Silver Solution, pour away, then add the rest of the solution and incubate for 15 min.
6. Wash briefly (approx 5 to 10 sec) with H_2O, twice.
7. Twice wash with a little Developing Solution, pour away, then add the rest of the solution and incubate until the bands are the desired intensity.
8. Pour off most of the developing solution, sprinkle solid citric acid into the solution containing the gel and swirl. Continue slowly adding the citric acid until the fizzing ceases.
9. Add a little H_2O, and incubate 15 min.
10. Incubate 3X 15 min with 3 changes of H_2O.

Destaining of Silver Stained Gels

Reference

Switzer, R.C., Merril, C.R., Shifrin, S. (1979). A highly sensitive stain for detecting proteins and peptides in polyacrylamide gels. Anal. Biochem. **98**: 231-237.

Reagents

Solution A:

0.633M sodium chloride (FW 58.44: 37 g/L)-0.231M cupric sulfate (FW 159.6: 37 g/L)

Add concentrated aqueous ammonium until the precipitate that forms is completely dissolved to give a deep blue solution.

Adjust volume to 1 liter with water.

Solution B:

1.75M sodium thiosulfate pentahydrate (FW 248.18: 4.36 g/10 ml)

Stop solution:

10% acetic acid

Procedure

1. Cut silver stained bands from the gel and put in individual microfuge tubes.
2. Prepare destain by combining equal parts of solutions A and B just prior to use.
3. Add 1 ml of destain to each tube.
4. One minute later add 300 µl of 10% acetic acid to each tube to stop the reaction.
5. Discard destaining solution from microfuge tubes.
6. Wash gels with water.