

Method for ABC Technique using Monoclonal Antibodies on Cultured Cells Grown on Glass Cover Slips

1. Cells are grown as monolayers on acid-washed, dH₂O-rinsed, sterile glass cover slips or slides. It may be necessary in some cases to coat cover slips or slides with poly L-lysine.
2. Wash attached cells in PBS for 5 minutes.
3. Fix cells in a fixative eg 1% v/v paraformaldehyde in PBS or Acetone or Zamboni's (the fixative of choice may depend on the antigen recognised) for 10 minutes.
4. Wash cells in PBS for 2 x 5 minutes.
5. Cover slips may be attached to glass slides using suitable adhesive, eg Loctite Glassbond, for convenience.
6. If required, permeabilise cells using 0.25% v/v Triton in PBS for 20 minutes.
7. Wash cells in PBS for 2 x 5 minutes.
8. Cover sections with blocking reagent, eg 10% normal rabbit serum in PBS, for 10 minutes.
9. Remove excess blocking reagent and replace with primary antiserum pre-diluted in blocking reagent for 60 minutes at 25°C or overnight at 4°C, according to the data sheet.
10. Rinse in PBS for 2 x 5 minutes.
11. Remove excess PBS and cover with biotinylated rabbit anti-mouse secondary diluted with blocking reagent for 30 minutes at 25°C.
12. Rinse in PBS for 2 x 5 minutes.
13. Remove excess PBS and cover with ABCComplex/HRP for 30 minutes at 25°C.
14. Rinse in PBS for 2 x 5 minutes.
15. Develop with 3 3' diaminobenzidine tetrahydrochloride (DAB).
16. Rinse slides in water.
17. Counterstain with Haematoxylin (if required).
18. Dehydrate, clear and mount sections with DPX mountant.