**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 25oC or overnight at 4oC, 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 25oC.

12. Rinse in PBS for 2 x 5 minutes.

13. Remove excess PBS and cover with ABComplex/HRP for 30 minutes at 25oC.

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.