

Method for ABC Technique using Polyclonal Antibodies on Paraffin Sections

1. Deparaffinize sections and rehydrate to distilled water.
2. Place sections in 0.5% v/v hydrogen peroxide/methanol for 10 minutes.
3. Pretreat slides for antigen retrieval using the appropriate method eg High Temperature Antigen Unmasking, trypsin etc, if required.
4. Wash slides with distilled water for 5 minutes.
5. Wash slides in 50mM Tris-Buffered Saline (TBS) pH 7.6 for 5 minutes.
6. Cover sections with blocking reagent eg 10% v/v normal swine serum in TBS for 10 minutes.
7. Remove excess blocking reagent and replace with primary antiserum diluted in blocking reagent as required (see datasheet), for 60 minutes at 25°C or overnight at 4°C.
8. Wash in TBS buffer for 2 x 5 minutes.
9. Remove excess TBS buffer and incubate sections with biotinylated swine anti-rabbit secondary diluted in blocking reagent for 30 minutes at 25°C.
10. Wash in TBS buffer for 2 x 5 minutes.
11. Remove excess TBS buffer and incubate sections with ABCComplex/HRP for 30 minutes at 25°C.
12. Wash in TBS buffer for 2 x 5 minutes.
13. Develop with 3 3' diaminobenzidine tetrahydrochloride (DAB).
14. Rinse slides in water.
15. Counterstain with Haematoxylin (if required).
16. Dehydrate, clear and mount sections with DPX mountant.