

Method for ABC Technique using Polyclonal Antibodies on Frozen Sections

1. Air dry frozen sections for 60 minutes or overnight.
2. Fix sections in acetone for 10 minutes.
3. Air dry sections for 10 minutes.
4. Wash sections in 50mM Tris buffered saline pH7.6 for 5 minutes.
5. Cover sections with blocking reagent eg 10% v/v normal swine serum in TBS for 10 minutes.
6. 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.
7. Wash in TBS buffer for 2 x 5 minutes.
8. Remove excess TBS buffer and incubate sections with biotinylated swine anti-rabbit secondary diluted in blocking reagent for 30 minutes at 25°C.
9. Wash in TBS buffer for 2 x 5 minutes.
10. Remove excess TBS buffer and incubate sections with ABCComplex/HRP for 30 minutes at 25°C.
11. Wash in TBS buffer for 2 x 5 minutes.
12. Develop with 3 3' diaminobenzidine tetrahydrochloride (DAB).
13. Rinse slides in water.
14. Counterstain with Haematoxylin (if required).
15. Dehydrate, clear and mount sections with DPX mountant.