

Method for ABC Technique using Polyclonal Antibodies on Paraffin Sections

The following method is recommended for rabbit polyclonal antibodies from Novocastra Laboratories Ltd. Customers should determine optimal dilutions for primary and secondary antibodies as these may vary according to the application.

REAGENTS

1. Standard solvents used in immunohistochemistry.
2. Hydrogen peroxide 30% w/v.
3. 50mM Tris buffered saline (TBS) pH7.6.
4. Normal swine serum.
5. Biotinylated secondary antibody.
6. ABC reagents.
7. DAB reagent.

EQUIPMENT

1. Appropriate incubators.
2. Standard immunohistochemistry facilities.
3. Pressure cooker or other appliance used for unmasking antigens - if required.

PROCEDURE

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.

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