Abnova Corporation

Immunohistochemistry Method

Deparaffinize sections and rehydrate using PBS.

Pre-treat the sample with **one** of the following procedures:

1. No treatment at all.
2. Place sample in 1X citrate buffer (pH 6.0) in pressure cooker under 125℃ for 4min and under 90℃ for 45min, cool sample subsequently. **(Method employed by Abnova)**
3. Place sample in 1X citrate buffer (pH 6.0) and microwave at 750W for 20 minutes, cool sample subsequently.
4. Place sample in 1X Tris/EDTA buffer (pH 9.0) and microwave at 750W for 20 minutes, cool sample subsequently.
5. Place sample in HCl (2N) (pH 0.6~0.9) at room temperature for 10~20 minutes.
6. Place sample in 0.1% trypsin and shake for 25 minutes at 37℃.

Step-by-step procedure:

1. Incubate sections in 3% H_2O_2 in 1X PBS at room temperature for 10 minutes and then wash the sections again.
2. Incubate sections in blocking solution for 10 minutes.
3. Add primary antibodies (diluted in blocking solution) and incubate the sections overnight at 4℃, wash sample with 1X PBS afterwards.
   (If using mouse MaxPab® primary antibodies, purification will be required, please see below)
4. Incubate sections with labeled polymer for 30 min followed by washing the sections with PBS.
5. Application of substrate solution (DAB or other suitable peroxidase substrate). Wash sample thoroughly under running tap water.
6. Counter stain the samples in Mayer's hematoxylin.
7. Dehydrate and mount samples.

Reagents:

- Blocking solution or Antibody Diluent (DAKO S3022)
- Immunodection Kit- EnVision Detection Kit, Peroxidase/DAB,Rabbit/Mouse (DAKO K5007)

- Citrate buffer, pH 6.0: 10 mM sodium citrate buffer
- 1X Tris/EDTA, pH 9.0: 10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20
- HCl solution (2N), pH 0.6~0.9: prepared in distilled water

Purification Protocols for mouse MaxPab®

1. Wash and equilibrate rProtein A Sepharose with 5 bed volumes of Protein A IgG Binding Buffer (Thermo Scientific), 3 times.
2. Load 50 uL sample containing IgG of interest into the micro-centrifuge tube containing 50 uL rProtein A sepharose, and add 200 uL of binding buffer, total volume 300 uL.
3. The sample and rProtein A sepharose were incubated on a 3D rocking platform at the room temperature for 2hrs.
4. Wash and equilibrate the spin column (Micro Bio-Spin Chromatography Column, Bio-Rad Laboratories) with IgG binding buffer.
5. Add the sample mixture from step 3 to the spin column.
6. Centrifuge at 2000 rpm for 5 seconds to collect flow through.
7. Wash column with 300 uL IgG binding buffer 3 times to remove unbound and non-specifically bound proteins.
8. Elute the bound IgG in 100 uL fractions into micro-centrifuge tubes containing 10 uL 1M Tris, pH 10 respectively. Collect at least 6 fractions.
9. Detect purified protein concentration by OD 280. Collect fractions containing purified protein.
10. Dialyze purified protein against PBS at 4℃.
11. Detect protein concentration by OD 280.
12. Store the purified IgG at -20℃.

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