

Protocol for immunohistochemistry used to test CD31 antibody

A: Solutions and Reagents

1. Phosphate buffered saline(pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na₂HPO₄ 4.3mmol/L, KH₂PO₄ 1.4mmol/L
2. Citrate buffer,0.01 M,pH6.0,Sodium Citrate 3g, Citric acid 0.4g
3. 3%Hydrogen peroxide
4. Primary antibody
5. Blocking serum (normal serum)
6. Biotinylated secondary antibody
7. DAB staining kit

B: Methods

1-Dewax and hydration slides through xylenes and EtOH

- 1) Dry slides for 20 min in oven at 60°C
- 2) Xylene,2×10'
- 3) 100%,95%,80%,70% EtOH,5'
- 4) Rinsed in PBS,5'

2-Antigen retrieval method (only for paraffin slides)

1: High-pressure antigen retrieval procedure (recommend method)

- 1) Place slides in a special glass slide holder and fill in the rest of the rack with slides to ensure even heating.
- 2) Rinsed rack in 1000 ml of Citrate buffer in ordinary pressure cooker,(0.01M,pH6.0),which could immerse all of glass slide.
- 3) Cover the cap and let heating for about 15-20 minutes on electric cooker until water boils(Steam ejected from pressure valve).Page 2
- 4) Begin timing,only 2 minutes in high-pressure
- 5) Remove the pressure cooker,Allow it to cool(Cool the slides at room temperature)
- 6) Rinsed the slides in PBS for 5 minutes

2: Microwave retrieval procedure

- 1) Place slides in a glass slide holder and fill in the rest of the rack with slides (10 total) to ensure even heating.
- 2) Rinsed rack in 1000 ml of Citrate buffer,(0.01M,pH6.0)
- 3) Microwave for 5X2 minutes
- 4) Cool the slides at RT

- 5) Rinsed the slides in PBS for 5 minutes
- 3-3% hydrogen peroxide solution treat, 10' at RT. PBS, 3X5'

- 4-Normal blocking serum treat, 20' at RT

- 5-Incubated with Primary Ab, 4°C overnight or 1.5 hours at 37°C

- 6-PBS, 3X5'

- 7-Biotin-conjugated second antibody, 20' at RT

- 8-PBS, 3X5'

- 9-Streptavidin-Peroxidase, 10' at RT

- 10-PBS, 3X5'

- 11-Staining with DAB solution, 2-5' under microscope

- 12-Stop the reaction by washing in tap water

- 13-Counterstain in Haematoxylin for 3-5 minutes

- 14-75%, 80%, 95% and 100% ethanol, 5×2', xylene 2×10'

- 15-Coverslide with Glycerin or Gum.