



Protocol for immunohistochemistry used to test VEGF 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).

- 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, 10' 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, 5x2', xylene 2x10'

15-Coverslide with Glycerin or Gum.

Please find all images for the VEGF antibody under “Additional Information”