

Protocol of Immunohistochemistry by Christina Scheffel

Proposed Kit:

DAKO EnVision™₊ System, Peroxidase (AEC) (DakoCytomation, cat.no. K4004, K4005, K4008, K4009)

Attention:

- Use a humid chamber for incubation steps!
- Perform the wash steps in glass-cuvettes
- This protocol is usable for cryosections and cytopins. Notice that
- cytopins should be fixed with paraformaldehyde before they will be frozen in -80°C!

1. Remove the slides out of -80°C. Air dry at RT for about 15 min.
2. Fix cryosections in 100% acetone for 10 min. in -20°C. Air dry the fixed sections for 30 min.
3. Make a circle around the sections with the Dako-pen (Dako-Pen from DakoCytomation, cat.no. S2002)
4. Wash: 1 x PBS1x/0,1%Tween 20, 30 sec. (PBS Dulbecco Powder, w/o Ca²⁺, Mg²⁺, Biochrom, cat.no. L182-50), (Tween20, Sigma, cat.no. P-7949),
5. Apply Peroxidase Block (Bottle 1), 1 drop, incubate 5 min. at RT
6. Wash: 1 x PBS1x/0,1%Tween 20, 30 sec.
7. Primary antibody: dilute to recommended concentration in Antibody Diluent (DakoCytomation, ChemMate, cat.no. S2022) about 100µL of the dilution per section/circle is necessary.
8. Incubate 30 min. at RT.
9. Wash: 3 x 5 min. PBS1x/0,1%Tween 20.
10. Secondary antibody: Labelled Polymer, HRP (Bottle 2). Apply 1 drop, incubate 30 min. at RT.
11. Wash: 3 x 5 min. in PBS1x/0,1%Tween 20.
12. Development: Apply ready-to-use AEC+ substrate-chromogen solution (Bottle 3).
13. Incubate 5-30 min.
14. Observe the color development under a microscope to prevent background staining!!!
15. After development put the slides back in PBS1x/0,1% Tween20
16. Counterstain: use Mayer's Haemalaun for microscopy (Merck, cat.no. 109249).
17. Incubate 1 min. at RT.
18. Wash: 3 x 5 min. with tap water.
19. Mount with DakoCytomation aqueous mounting medium (DakoCytomation, cat. no. S3025): 1 section per slide - use 1 drop of mounting medium and a coverslip with the size 24x30mm; 2 sections per slide - use 1 drop per section and use a coverslip with the size 24x60 mm
20. Storage: Air dry the mounting medium. After documentation store slides in special "slide boxes" at room temperature.

RT = room temperature