

# Immunoperoxidase Protocol For IHC (Immunohistochemistry)

## Day One:

- 1- Dewax in xylene for **-10 min 2 x**
- 2- Rehydrate 100% alcohol **-10 min**
- 3- 90% alcohol **- 10 min**
- 4- 70% alcohol **- 10 min**
- 5- Wash in PBS (**5min**)
- 6- Triton (0.2% in PBS) **-30 min.** at R.T.
- 7- PBS (**5min –3 x**)
- 8- H<sub>2</sub>O<sub>2</sub> (5% in PBS) **30 min** . at RT
- 9- PBS (**5 min 3 x**)
- 10-Block by using Universal blocking Solution (Dako) **20-30min** at RT.
- 11-Primary AB diluted in AB diluting buffer ( Dako)incubate at 4 C overnight.

## Day Two:

- 1- Drain Ab and wash with PBS for **5 min 3 x**.
- 2- prepare 2<sup>nd</sup> biotinylated Ab --Apply and incubate for **45 min.** at R.T.
- 3-Prepare ABC complex: 1 ml PBS+ 10 mul sol. A+ 10 mul sol B- **30 min.** in advance.
- 4- Wash with PBS **5min 3 x**.
- 5- Add ABC complex incubate **45 min** at RT.
- 6- Wash with PBS **-5 min. 3 x**
- 7- Prepare DAP developing stain (20ul in 1ml buffer solution). Careful, it is carcinogenic!!
- 8- Develop color and observe using a microscope. Approximately **2-3 min.**
- 9- Wash with PBS, followed by a wash in tapwater.
- 10- Counterstain with Haematoxylin **-3 seconds maximum.**
- 11- Wash in tap water.
- 12- Blue in Saturated Lithium Carbonate for **20 sec.**
- 13- Wash in tap water.
- 14- Dehydrate 70%, 90%, 100% Ethanol and Xylene
- 15- Coverslip with Cytoseal 60 and airdry.

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