Immunohistochemistry (IHC)

Procedure

1. Mount and section samples
2. Heat sections on the specimen slide to improve adherence. Put the specimen slide in the water bath and heat at 65°C 30 min.
3. Remove paraffin and rehydrate the tissue

   Place the slides in a rack, and perform the following washes:

   a. Xylene: 3 x 3 minutes.

   b.100% ethanol: 3 minutes.

   c. 95% ethanol: 3 minutes.

   d. 85 % ethanol: 3 minutes.

   e. 70 % ethanol: 3 minutes.

   f. Running cold tap water to rinse.
4. Perform heat induced or protease induced epitope retrieval

   Immerse slides into preheated **Antigen Retrieval Solution** (1x, 100°C) 10 minutes. Remove slides from the water bath, and let it cool to room temperature. Gently rinse the slides with PBS 3 times (3 minutes each time).
5. Block endogenous peroxidases, phosphatases (for enzymatic labels) and **biotin** (when using biotin/avidin systems) Put the slides in 3% H_2O_2-methanol solution (30% H_2O_2: 100% methanol=1: 9) at room temperature 15 min, and gently rinse the slides with PBS 3 times (3 minutes each time).
6. Block non-specific binding sites

   Put the slides in 5% non-fat milk at 37°C 5 min, and gently rinse the slides with PBS 3 times (3 minutes each time).
7. Incubate with primary antibody

   Put the slides in the wet box, and add proper volume of primary antibody diluent, then incubate at 37°C 60 min, and gently rinse the slides with TBST 3 times then PBS (3 minutes each time).
8. Incubate with secondary antibody

   Add proper volume of secondary antibody diluent, then incubate at 37°C 10 min, and gently rinse the slides with TBST 3 times then PBS (3 minutes each time).
9. Incubate with amplification reagent

Add proper volume of **avidin diluent**, then incubate at 37°C 10 min, and gently rinse the slides with TBST 3 times then PBS (3 minutes each time).

When performing experiments with multiple fluorescent labels, ensure that each fluorophore can be spectrally separated. This ensures that one fluorophore does not get detected in another fluorophore's channel. For this purpose, we recommend mocking up the fluorophore excitation and emission spectra with the help of a spectrum viewer at the experimental design stage.

10. Incubate with DAB or other substrate solution (for enzymatic labels only)

    Add 1–5 drops of **DAB chromogen solution** to cover the entire tissue section and incubate for 3–5 minutes, and rinse in deionized H_2O and drain the slides.

11. Counterstain

    Add 1–5 drops of hematoxylin to cover the entire tissue section and incubate for 5 minutes, and rinse in deionized H_2O and drain the slides.

12. Dehydrate tissue sections (only needed when organic mounting media are used)

    Cover stained tissue with a coverslip of an appropriate size. Place slides vertically on a filter paper or towel to drain excess mounting medium and allow them to dry.

13. Mount coverslip

    Add drops of resinene, then seal with coverslip. Visualize tissue under a light microscope.