Immunohistochemistry (IHC) Staining Protocol

1. **Rehydration**: Rehydrate tissues before starting DAB (3,3′ -diaminobenzidine tetrahydrochloride) staining.

a. Immerse slides in xylene (mixed isomers) twice, 10 min each.

b. Immerse slides in 100% ethanol twice, 10 min each.

c. Soak slides in 95% ethanol for 5 min.

d. Soak slides in 70% ethanol for 5 min.

e. Soak slides in 50% ethanol for 5 min.

f. Rinse slides with deionized water.

g. Rehydrate slides in wash buffer for 10 min, then drain excess buffer.

2. **Peroxidase Blocking**: To inhibit endogenous peroxidase activity, incubate samples with 1–3 drops of peroxidase blocker (3% H_2O_2 in water or methanol) for 15 min.

3. **Washing**: Rinse slides with PBS 3 times (1 min each), then drain.

4. **Antigen Retrieval**: Place the slides in antigen retrieval solution (usually a citrate buffer at pH 6.0), and heat in a microwave oven or autoclave to re-expose the antigenic determinants masked during fixation.

5. **Washing**: Rinse slides with PBS 3 times (1 min each), then drain.

6. **Hydrophobic Barrier** (Optional): Use a barrier pen to surround the tissue with the hydrophobic barrier.

7. **Blocking**: Apply blocking solution(5% BSA) and incubate at room temperature (RT) for 30 min.

8. **Washing**: Rinse slides with PBS 3 times (1 min each), then drain.

9. **Primary Antibody Incubation**: Remove blocking solution, apply primary antibody, and incubate at 37°C for 2 hr or at 4°C overnight.

10. **Washing**: Rinse slides with PBS 3 times (1 min each), then drain.

11. **Secondary Antibody Incubation**: Apply secondary antibody and incubate at RT for 30–60 min.

12. **Washing**: Rinse slides with PBS 3 times (5 min each), then drain.

13. **DAB Development**: Add 1–5 drops of DAB substrate to fully cover the tissue section. Incubate for 5–10 min. Monitor staining intensity under a microscope. A colored precipitate forms at antigen sites as HRP converts DAB into an insoluble product.

Caution: DAB is hazardous. Wear gloves, safety goggles, and work in a fume hood. Follow MSDS guidelines.

Note: If necessary, DAB enhancer can be used to enhance the DAB chromogen solution.

14. **Washing**: Rinse slides with PBS 3 times (5 min each), drain, then rinse with deionized water and drain.
15. **Counterstaining**: The stained tissue can either be left without nuclear counterstaining, or counterstained with hematoxylin nuclear counterstaining to better observe the tissue morphology.
16. **Microscopy**: Apply a coverslip of appropriate size, remove excess liquid, and observe staining under a microscope.