



General IHC Protocol

This protocol is intended as a general guide, users should determine optimal conditions for each procedure. Use all appropriate laboratory safety precautions when following this protocol.

Frozen Sections

1. Fresh tissues should be snap-frozen in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryo molds. Store frozen blocks at -80 deg C.
2. Cut 4-8 mm thick cryostat sections, mount sections on Superfrost plus slides or gelatin coated slides. Store slides at -80 deg C until required.
3. Prior to staining, allow slides to reach room temperature (approx 30 minutes) then fix in ice-cold acetone for 10 minutes. Air dry for a further 30 minutes.
4. Wash slides in PBS buffer.

Paraffin Sections

1. Deparaffinize slides containing the sections in xylene twice for 5 minutes each time.
2. Hydrate sections twice with 100% ethanol for 3 minutes each time.
3. Hydrate sections with 95% ethanol.
4. Rinse sections in ultra pure water.

Procedure for Immunoenzyme Staining

1. Follow appropriate pretreatment procedure if required.
2. Rinse sections twice in PBS buffer for 2 minutes each time.
3. **Serum Blocking:** Incubate sections in normal serum block appropriate to the secondary antibodies host species (for example, Normal Goat Serum if secondary antibody was raised in Goat)

Note:

Since this protocol uses an avidin-biotin detection system, avidin/biotin block may be needed based on tissue type. If you do, the avidin/biotin blocking should be performed now after the normal serum blocking and before the primary antibody incubation.

5. **Primary Antibody:** Incubate sections in primary antibody at an appropriate dilution in dilution buffer (PBS supplemented with serum block) for 1 hour at room temperature or overnight.

*Note: Do not rinse sections between serum block and primary antibody incubation.
Rinse in PBS buffer 3 times for 2 minutes each time.*

6. **Peroxidase Blocking:** Incubate sections in 1% hydrogen peroxidase in PBS buffer for 10 minutes at room temperature.
7. Rinse in PBS buffer 3 times for 2 minutes each.
8. **Secondary Antibody:** Incubate sections in biotinylated secondary antibody (1:500) in PBS buffer for 30 minutes at room temperature.
9. Rinse in PBS buffer 3 times for 2 minutes each.
10. **Detection:** Incubate sections in streptavidin-HRP in PBS buffer for 30 minutes at room temperature.
11. Rinse in PBS buffer 3 times for 2 minutes each.
12. **Chromagen/Substrate:** Incubate sections in peroxidase substrate solution for stable blue or brown staining, respectively.
13. Rinse in PBS buffer 3 times for 2 minutes each.
14. Rinse in ultra pure water 3 times for 5 minutes each.
15. Dehydrate through 95% ethanol for 1 minute, then 100% ethanol twice for 3 minutes each time.
16. Clear in xylene twice for 5 minutes each time.
17. Apply coverslip with appropriate mounting medium.