NEUROMICS

Sample Immunohistochemistry Protocol (Paraffin Sections)

1. Fixation and Sectioning
    a. Fix dissected tissues in 2% paraformaldehyde, Bouin's solution, or other fixative for 30 minutes to overnight. (NOTE: Fixation time will depend on species, tissue origin and size of tissue sections).
    b. Embed tissue in paraffin.
    c. Section the tissues 5-10 micrometers thick.
    d. If using parafomaldehyde as fixative antigen retrieval may be necessary.

2. Deparaffinization and Tissue Rehydration
    a. Deparaffin samples by incubating sections 2-3 times in xylene for 10 minutes each.
    b. Hydrate samples by placing twice in 100% ethanol for 3 minutes each, then in 95%, 70%, 50%, 30% ethanol for 2 minutes each.

3. Blocking and Primary Antibody Incubation
    a. Block slides with 10% serum from the species from which the secondary antibody was taken or 8% BSA.
    b. Incubate for 30 minutes to 1 hour at room temperature in a humidified chamber.
    c. Wash samples in PBS containing 2% BSA for 5 minutes.
    d. Incubate slides in a humidified chamber overnight with primary antibody solution (primary antibody in PBS containing 2% BSA).
    e. Wash three times with PBS containing 2% BSA for 5 minutes.

4. Secondary Antibody Incubation and Detection
    a. Incubate slides in a humidified chamber with secondary antibody solution (secondary antibody in PBS containing 2% BSA) according to manufacturer's protocols.
    b. Wash three times with PBS containing 2% BSA for 5 minutes.
    c. Detect according to manufacturer's protocols.