

## Sample Immunohistochemistry Protocol (Frozen Sections)

### 1. Fixation and Sectioning

- a. Freeze and section tissue samples.
- b. Fix sections in Bouin's solution, -20°C acetone, -20° C methanol, or formaldehyde for 2-30 minutes. (NOTE: Fixation time will depend on species, tissue origin and size of tissue sections).

### 2. Blocking and Primary Antibody Incubation

- a. Sections are rinsed in 0.1% Triton in phosphate-buffered saline solution (pH 7.4, PBST) for 30 minutes.
- b. Block slides with 5% serum from the species from which the secondary antibody was taken in PBST..
- c. Incubate for 30 minutes to 1 hour at room temperature in a humidified chamber.
- d. Wash samples in PBST containing 2% BSA for 5 minutes.
- e. Incubate slides in a humidified chamber overnight with primary antibody solution (primary antibody in PBST containing 2% BSA).
- f. Wash three times with PBST containing 2% BSA for 5 minutes.

### 3. Secondary Antibody Incubation and Detection

- a. Incubate slides in a humidified chamber with secondary antibody solution (secondary antibody in PBST 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.