Immunohistochemistry Protocol - Mouse Antibodies That Do Not Require Antigen Retrieval.
(4% Paraformaldehyde- & Formalin-Fixed Paraffin Embedded Tissue)

Specimen preparation:

1. Dewax sections through 3-4 changes of xylene and rehydrate through descending alcohol to water.
2. Soak in PBS for 5 min.
3. Quench endogenous peroxidase for 15 min at room temperature with 1.5% hydrogen peroxide in methanol.

Pretreatment:

Blocking: For any monoclonal antibody use on mouse tissues, block using a MOM kit (Vector labs). For use on human tissue, use 2% normal horse serum in PBS + 0.2% Triton X-100 (normal serum block) for 2 hr at room temperature to block.

Staining procedure (for human tissue):

1. Incubate samples overnight at 4^{o}C with the primary antibody diluted in normal serum block (2-4% normal horse serum in PBS/0.2% Triton X-100) at the dilution indicated.
2. Wash sections 5 times for 5 min each in PBS containing 0.2% Triton X-100.
3. Incubate 30 min at room temperature with biotinylated horse anti-mouse antibody diluted 1:200 in blocking solution.
4. To detect antigen:antibody complexes we recommend using a Vectastain ABC Peroxidase Elite Mouse IgG kit as follows (or use your own detection system):
    1. Incubate sections for 30 min at room temperature with avidin/biotin/peroxidase complex (ABC kit from Vectastain) diluted in blocking solution.
    2. Incubate with NiDAB in 0.1 M acetate buffer for 4 min at RT.
    3. Incubate with Tris cobalt for 4 min at RT.
    4. Counterstain with nuclear fast red for 2 min at RT.

Staining procedure (for mouse tissue):
Follow directions for the Vector Labs MOM (Mouse On Mouse) kit.

Controls:
Controls include

1. Mouse IgG or nonimmmune sera at the same dilution as the primary antibody
2. Omission of the primary antibody to check for endogenous biotin and peroxidase activity, as well as nonspecific binding of the secondary antibody.