Immunohistochemistry Protocol - Rabbit Antibodies That Do Not Require Antigen Retrieval

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

Pretreatment:

Antigen recovery/retrieval, (required for any nuclear antigen): Immerse sections in 10 mM sodium citrate, pH 6.0, and heat in a microwave for 15 min at 90^{o}C.

Blocking: Block sections in 2-4% (unless otherwise indicated) normal serum* in PBS + 0.2% Triton X-100 (normal serum block) for 2 hr at room temperature.

*NOTE: serum should be from the same source that the secondary antibody is derived from.

Staining procedure:
Incubate samples overnight at 4^{o}C with the primary antibody diluted in normal serum block (2-4% normal goat serum in PBS/0.2% Triton X-100) at the dilution indicated. Wash sections 5 times for 5 min each in PBS containing 0.2% Triton X-100. Incubate 30 min at room temperature with biotinylated anti-rabbit secondary antibody diluted 1:200 in blocking solution.

To detect antigen:antibody complexes use a Vectastain ABC Peroxidase Elite Rabbit IgG kit as follows: Incubate sections for 30 min at room temperature with avidin/biotin/peroxidase complex (ABC kit from Vectastain) diluted in blocking solution, then NiDAB in 0.1 M acetate buffer for 4 min at RT, then Tris cobalt for 4 min at RT. Counterstain with nuclear fast red for 2 min at RT.

Controls:
Controls include 1) preimmune and/or nonimmune rabbit sera or rabbit IgG 2) omission of the primary antibody to check for endogenous biotin and peroxidase activity, as well as nonspecific binding of the secondary antibody.