Immunohistochemical staining

Tissue sections were dewaxed with xylene and rehydrated through gradient ethanol into water. For antigen retrieval, sections were heated in citrate buffer (pH6.0) for 10 min at 95°C in a microwave oven. After cooling to room temperature, the sections were then digested with 0.05% trypsin for 10 min at 37°C. Endogenous peroxidase activity was quenched with 0.3% H_2O_2 in methanol for 30 min at room temperature. After PBS washes, nonspecific antibody binding was blocked by preincubating slides with 10% normal goat non-immune serum at 37 ° for 30 min. After blotting off the blocking serum, sections were incubated with diluted primary antibody at 4℃ overnight. After PBS washes again, sections were incubated with biotinylated secondary antibody at 1:200 dilution for 30 min at room temperature. After incubating with Vectastain ABC reagent (Vector Laboratories, Inc., Burlingame, CA) for 30 min at room temperature, the sections were developed with DAB (Sigma-Aldrich, St. Louis, MO). Sections were washed in running tap water and lightly counterstained with hematoxylin, followed by dehydration and coverslip mounting. Negative controls were obtained by omitting the primary antibody.