Anti-human Prox-1: Protocol for IHC

Day 1
Dewax and rehydrate slides
Boil in boiling buffer 10 min in pressure cooker
Cool down slowly to RT in boiling buffer
Wash 3 x 5 min in TNT
Block 45 min in TNB
Incubate with Prox1, diluted 1:200 in TNB, o/n at +4 C

Day 2
Wash 3 x 5 min in TNT
Incubate 1h at RT with biotinylated anti-rabbit antibody, diluted 1:100 in TNB
Wash 3 x 5 min in TNT
Incubate 45 min RT with SA-Alexa fluor 568, diluted 1:100 in TNB
Wash 3 x 5 min in TNT
Visualize

Solutions
Boiling buffer (1L, 50xstock)
242 g Tris base
18.6 g EDTA

Working solution:
Dilute 5 ml of stock solutionj to 250 ml. Adjust pH to 8 by adding approximately 250 mkl of conc. HCl

TNT
0.1 M Tris-HCl, pH 7.5
0.15 M NaCl
0.05% Tween-20

TNB
0.1 M Tris-Hcl, pH 7.5
0.15 M NaCl
0.5% Blocking reagent (supplied in TSA Biotin System kit NEL700, NEN Life Science Products)

Protocol II

This protocol has been used by some of my colleagues (Dr. Rui M. Reis, School of Health Sciences, University of Minho, Braga, Portugal) with formalin-fixed, paraffin-embedded tissue blocks.

Paraffin Sections were immersed in antigen retrieval solution (DAKO, Glostrup, Denmark) and microwaved at 600w for 20 minutes. Sections were further incubated with Ultravision block solution (Neomarkers, Freemont, CA, USA) for 10 minutes at room temperature before incubating 60 minutes at room temperature with the first primary antibody (Prox-1, 1:1000). Sections were sequentially washed in PBS and incubated with biotinylated goat anti-polyvalent immunoglobulin for 10 minutes, streptavidin-peroxidase for 10 minutes, and developed with 3,3'-diamino-benzidine for 10 minutes. Slides were mounted in aquatex medium (Merck, Dermstadt, Germany).