

Materials and Methods for immunohistochemistry:

The paraffin embedded sections were cleared and the sections were incubated with 0.1% Pronase (Roche #165 921) in 0.1% CaCl₂ pH 7.8. at 37°C for 10 minutes. They were blocked with 3% H₂O₂ in TBS for 10 mins., washed then blocked with Dako Biotin Blocking System (Dako X0590). After washing, they were further blocked with 10% Normal Rabbit Serum for 10 mins. at room temperature (RT) and incubated firstly with 1/800 (1.25µg/ml) Hb65 antibody for 1hr at RT, then with biotinylated Rabbit Anti-Mouse (Dako, E-0354) at 1/100 for 30 mins. at RT, and finally with Strep-ABC complex (Dako, K-0377) at 1/100 for 30 mins. at RT. The sections were developed with AEC substrate kit (vector lab, SK-4200) at RT for 20 mins., counterstained with haematoxylin and after drying were mounted with DAKO aqueous mount (Dako, 003181).