Protocol of IHC

For research use only, not for use in diagnostic procedures.

Immuno-Biological Laboratories Co., Ltd.

Anti-Human Gd-IgA1(KM55) Rat IgG MoAb

① Paraffin-embedded sections of 3µm thickness
② Deparaffinization with a series of xylene and ethanol concentrations and subsequent rehydration
   Xylene 10min. x2
   100% Ethanol 5min. x3
   90% Ethanol 5min. x1
   70% Ethanol 5min. x1
   50% Ethanol 5min. x1
   Rinse with distilled water 5min

③ Antigen retrieval : 0.05% bacterial protease subtilisin A (Sigma-Aldrich, Tokyo, Japan) dissolved in 5mmol/l tris (hydroxymethyl)-aminomethane buffer (pH7.6) room temp. 2hr
   *This process is extremely important. If the dose of bacterial protease subtilisin A is not enough, KM55 mAb staining will not be succeeded.

④ Rinse with distilled water.
⑤ Block with Protein Block (DAKO, Tokyo, Japan). room temp. 30min
⑥ 1st Ab : Anti-Human Gd-IgA1(KM55) Rat IgG 100µg/mL 37°C 60min
⑦ Several washes with phosphate-buffered saline and triethanolamine-buffered saline containing 0.05% tween-20.
⑧ 2nd Ab: Alexa Fluor 555(or Other colors)-conjugated goat anti-rat IgG antibody (1:1000; Life Technologies, Carlsbad, CA). 37°C 30min
⑨ Several washes with phosphate-buffered saline and triethanolamine-buffered saline containing 0.05% tween-20.
⑩ Seal with Fluoromount (Diagnostic BioSystems, Pleasanton,CA).