IF and IHC staining of type IV collagen in Alport's Syndrome

Mouse anti human collagen IV a1 Chain
Mouse anti human collagen IV a3 Chain
Mouse anti human collagen IV a5 Chain

Clone names: 4E5E2-1 (mouse IgG1/kappa), & 18G6-2 (mouse IgG1/kappa), & 8A12D2-2

Quantity: 0.1ml

Application: WB, IHC, IF

Physical state: Liquid

Specificity of antibodies: 4E5E2-1 is specific to NC1 domain of α3(IV) ; 18G6-2 is specific to NC1 domain of α5(IV); 8A12D2-2 is specific to NC1 domain of α1(IV).

Preparation of antibodies : Monoclonal antibodies were prepared by the mouse splenocytes fusion method developed by GenScript Biotechnology Co., Ltd. with synthetic peptides NC1 domain of type IV collagen as immunogens.

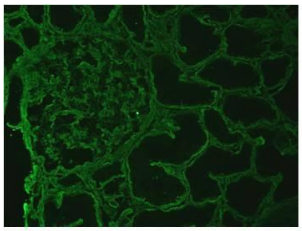
Appearance: Solution, Monoclonal antibodies were purified by affinity chromatography. 0.02% ProClin 300 is added for preservation.

KIT COMPONENTS AND STORAGE OF REAGENTS

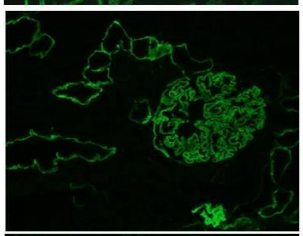
100ul mouse monoclonal antibody to alfa 1 chain of type IV collagen
100ul mouse monoclonal antibody to alfa 3 chain of type IV collagen
100ul mouse monoclonal antibody to alfa 5 chain of type IV collagen
1x 10ml glycine/urea solution

For research use only. Not for use in diagnostic procedures.

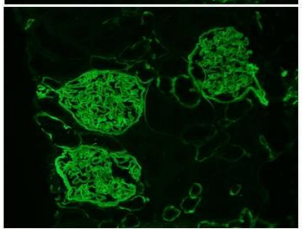
Alport's syndrome, an inherited disease, shows absence or reduction of the a5 chain in the glomerular , tubular and bowman's capsular basement membranes. Normal human kidney has a1(IV) to a6 (IV) chains in the renal basement membrance.



FITC fluorescence a1 chain is observed in the GBM and TBM



FITC fluorescence a3 chain is observed in the GBM and part of the TBM



FITC fluorescence a5 chain is observed in the GBM , part of the TBM and Bowman's capsular

METHOD1：cryostate sections

1. For each tissue specimen to be analyzed, cut three 3 um thick cryostate section, air dry and fix in acetone for 10 minutes.
2. The section to be stained with a1、a3 and a5 are incubated in the glycine/urea solution (provided with the kit) for 8 min at room temperature.
3. Wash the three slides in PBS for 5 minutes and leave the slides in PBS.
4. Incubate the three tissue slide for 1 hour with their respective monoclonal antibody:
    a.Mouse anti human collagen IV a1 Chain diluted 1:50.
    b. Mouse anti human collagen IV a3 Chain diluted 1:50.
    c. Mouse anti human collagen IV a5 Chain diluted 1:50.
5. Wash in sterile PBS for 10 minutes.
6. Incubate the three tissue slides for 1 hour with their respective FITC-labelled secondary antibodies.
7. Wash in sterile PBS for 10 minutes and add mounting media containing.

METHOD2：Paraffin embedded tisue sections

1. For each tissue specimen to be analyzed, cut four 3-4 um thick formalin-fixed,paraffin-embedded tissue sections,deparaffinise and rehydrate.
2. Antigen demasking: microwave boiling in citrate buffer, PH 6 at 750W for 10 min followed by 350W for 15 min.
3. Rinse the four slide in distilled water for 2 minutes. the section to be stained with a1、a3、a5 and negative control slides are incubated in the glycine/urea solution (provided with the kit) for 10 min at room temperature.
4. Rinse in distilled tap water for 2min.
5. Endogenous peroxidases are blocked by incubating the four slide with 1.5% H2O2 in TBS for 10min.
6. Rinse the five slides in tap water for 5min,followed by distilled water for 1 minute
7. Wash the four slides in PBS for 5 minutes and leave the slides in PBS.
8. Incubate the three tissue slide for 1 hour with their respective monoclonal antibody:
    a. Mouse anti human collagen IV a1 Chain diluted 1:50.
    b. Mouse anti human collagen IV a3 Chain diluted 1:50.
    c. Mouse anti human collagen IV a5 Chain diluted 1:50.
    d. Negative control
9. Rinse the four slides in PBS for 5 minute
10. Finalise the staining of the four tissue slides according to the instructions of your respective detection systems.