COSMO BIO CO., LTD.
Inspiration for Life Science

Engl. 060523

MONOCLONAL ANTIBODIES PRODUCED
BY THE RAT LYMPH NODE METHOD

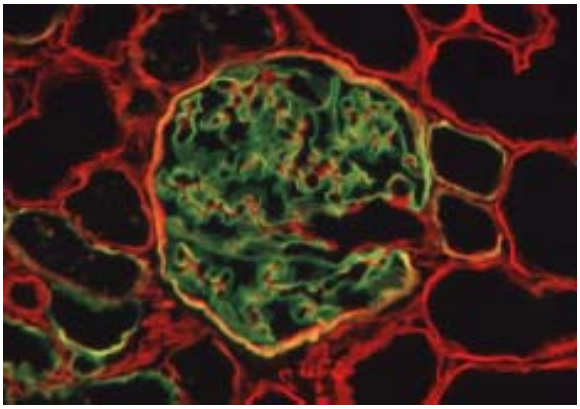
For research use only

Fluorochrome-conjugated MoAbs for Alport's syndrome

FITC-Anti Collagen IV α5(IV) Chain, Human (Mono)
+ Texas Red-Anti Collagen IV α2(IV) Chain, Human (Mono)

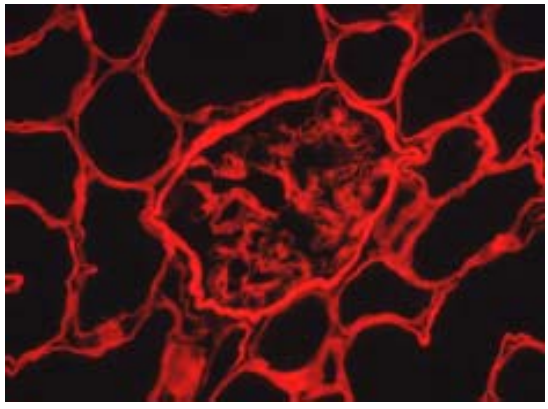
Code: SGE-CFT45325 **Quantity: 1.0 ml**

This product can be used for staining of human renal and skin biopsy sections very easily and rapidly because two monoclonal antibodies are conjugated with two different fluorochromes. FITC-conjugated-anti α5(IV) demonstrates presence or absence of the α5(IV) chain, and Texas Red -anti α2(IV) reveals the renal basement membrane structure.
Alport's syndrome, an inherited disease, shows absence or reduction of the α5(IV) chain in the glomerular, tubular and Bowman's capsular basement membranes. Normal human kidney has α1(IV) to α6(IV) chains in the renal basement membranes.



Double exposure of normal human kidney section stained with the product.

FITC fluorescence (α5 chain) is observed in the GBM, part of the TBM and Bowman's capsular BM. BMs were two fluorochromes are present look orange to yellow.



Double exposure of Alport human kidney section stained with the product.

Because no FITC fluorescence (α5 chain) is observed in the kidney of the patient with X-linked Alport's syndrome, only Texas Red fluorescence (α2 chain) is confirmed.

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Quantity: 1.0 ml

Appearance: Solution. Monoclonal antibodies were purified by affinity chromatography. The monoclonal antibodies against α5(IV) were conjugated with FIFC (H53 & B51), and the monoclonal antibody against α2(IV), with Texas Red (H25). 0.1% NaN_3 is added for preservation.

Clone names: H53 (rat IgG2a/kappa, B51 (rat IgG2a), & H25 (rat IgG1/kappa)

Specificity of antibodies: H53 is specific to imperfection III of α5(IV) (Reference 1); B51 is specific to NC1 domain of α5(IV) (Reference 2); H25 is specific to imperfection XIII of α2(IV) (Reference 1).

Preparation of antibodies : Monoclonal antibodies were prepared by the rat lymph node method developed by Shigei Medical Research Institute with synthetic peptides and native NC1 domain of type IV collagen as immunogens.

use : Staining of human cryostat sections by direct immunofluorescence (No acid-urea treatment is necessary.)

Storage : In dark at 2-4℃, or in freezing at below -30℃. Stable at these conditions for several years

Reference : 1) Kagawa et al. (1997) Nephrol. Dial. Transplant. 12: 1238-1241
2) Borza DB et al. (2001) J. Biol. Chem. 276: 28532-28540

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How to use SGE-CFT45325

● SGE-CFT45325 is prepared to stain human cryostat sections of renal and skin biopsy specimens by direct immunofluorescence.

How to stain human kidney cryostat sections

Just put 20-50 µl of the staining solution on cryostat sections, incubate them for 30 min at room temperature. After washing them with PBS, mount them and observe them with a fluorescence microscope.

Caution: Do no use stale cryostat sections.
Do not fix sections with any fixatives.
Do not dilute the staining solution.

How to stain human skin cryostat sections

After acetone treatment of cryostat sections for 5 min, put 20-50 µl of the staining solution on them, and incubate them for 60 min in a moisture chamber at room temperature. After washing them with PBS, mount them and observe them with a fluorescence microscope.

Caution: Do no use stale cryostat sections.
Do not fix sections with any fixatives.
Do not dilute the staining solution.

● In case that a section is not stained or stained in a mosaic pattern with FITC-anti-α5(IV) but the background staining of Texas Red-anti- α2(IV) is normally stained
The disease is diagnosed as Alport's syndrome.

Caution: Human skin basement membrane is a thin membrane and sometimes staining of α5(IV) is very weak.

For accurate diagnosis, it is important to consider clinical symptoms. In case that the staining is not clear, it is necessary to make additional staining with other monoclonal antibodies against type IV collagen.

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References:

1) Kagawa M et al. (1997) Epitope-defined monoclonal antibodies against type-IV collagen for diagnosis of Alport's syndrome. Nephrol. Dial. Transplant. 12: 1238-1241.
2) Yoshioka K et al. (1994) Type IV collagen a5 chain: Normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibody. Am. J. Pathol. 144: 986-996.
3) Ninomiya Y et al. (1995) Differential expression of two basement membrane collagen genes, COL4A6 and COL4A5, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies. J. Cell Biol. 130: 1219-1229.
4) Naito I et al. (1996) Relationship between COL4A5 gene mutation and distribution of type IV collagen in male X-linked Alport syndrome. Kidney Int. 50: 304-311.

Distributor
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