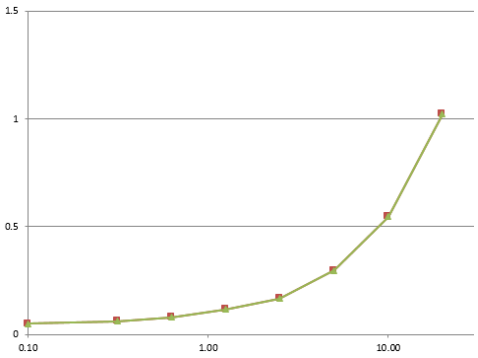
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C5b-9, Rat, clone 2A1		
Catalog number	HM3033-100UG		
Lot number	-	**Expiry date**	-
Volume	1 ml	**Amount**	100 µg
Formulation	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3	**Concentration**	100 µg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	N.A.	**Purification**	Protein G
Storage	4°C		

Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	2,3,6-8	8	1,5,9	4		1,10		
Yes	●	●	●	●		●		
No								●
N.D.					●		●	

N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



IA: Immuno assay experiment with HM3033. HM3033 was used as a capture antibody.

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

- IA: plates were coated with 30µg/ml in 50mM carbonatebuffer, pH10.6 for 16h at 4°C.(Ref.1)
- W: The band size of TCC is too large for western blot (>200 kDa)
- IHC-P: Tissue sections fixed in formalin were pretreated with protease type XXIV for 10 minutes at 37ºC before incubation (Ref.6).
- IHC-F: Tissue sections were fixed in acetone for 10 minutes at room temperature before incubation (Ref. 3).

General Information

Description

The monoclonal antibody 2A1 recognizes rat C5b-9. The antibody was shown to compete with antibodies to human C9 for its binding site on the C5b-9 complex, indicating that the reactive epitope is located on the C9 molecule. C5b-9 membrane attack complexes are assembled from five precursor molecules in the serum. Proteolytic cleavage of C5 by C5 convertase generates C5b which initiates assembly of the C5b-9 complex. The last step of C5b-9 complex formation involves polymerization of C9 which accompanies insertion of the complex into the cell membrane. During formation of C5b-8 and C9 polymerization, neoantigens are generated which are unique to the C5b-9 complex and are not present on any of the individual native complex components. The complement regulatory proteins CD59 and complement S-protein can both prevent C5b-9 insertion into the cell membrane. The formed SC5b-9 complex is unable to attach to cells and is cytolytically inactive.C5b-9 is involved in the progression of chronic proteinuric renal disease by mediating continuous tubulointerstitial damage. Early tubulointerstitial injury in the remnant kidney can be improved when C5b-9 complex forming is abrogated. The monoclonal antibody 2A1 was raised against a rat C5b-9 neoantigen. Monoclonal antibody 2A1 can be used as a coating antibody to detect C5b-9 in plasma and urine samples.

Immunogen

Rat C5b-9

Aliases Membrane attack complex, MAC, TCC, Terminal Complement Complex

References

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2. Brandt, J et al. Role of the complement membrane attack complex (C5b-9) in mediating experimental mesangioproliferative glomerulonephritis. Kidney Int. 1996, *49*: 335
3. Sato, T et al.The terminal sequence of complement plays an essential role in antibody-mediated renal cell apoptosis. J Am Soc Nephrol 1999, *10*: 1242
4. Duijvestijn, A. Complement activation by anti-endothelial cell antibodies in MHC-mismatched and MHC-matched heart allograft rejection: anti-MHC, but not anti non-MHC alloantibodies are effective in complement activation. Transpl. Int. 2000, 13:363
5. Yamada,K et al. Clusterin is up-regulated in glomerular mesangial cells in complement-mediated injury. Kidney Intern 2001. 59:137
6. Nangaku M et al. C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys J. Am. Soc. Nephrol. 2002, *13*: 928
7. Rangan GK et al. C5b-9 regulates peritubular myofibroblast accumulation in experimental focal segmental glomerulosclerosis. Kidney Int 2004, *66*: 1838
8. Ostendorf, T et al. Antagonism of PDGF-D by human antibody CR002 prevents renal scarring in experimental glomerulonephritis. J Am Soc Nephrol 2006, *17:* 1054
9. Pol, P; Pathogenic role of complement in renal ischemia/reperfusion injury. Thesis 2013
10. Kotimaa, J et al; Functional assessment of rat complement pathway activities and quantification of soluble C5b-9 in an experimental model of renal ischemia/reperfusion injury. J Imm Meth 2014, *412*: 14

Storage&stability Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

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Approved by Manager of QC	Date
Brenda Teunissen	28/09/2021

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.