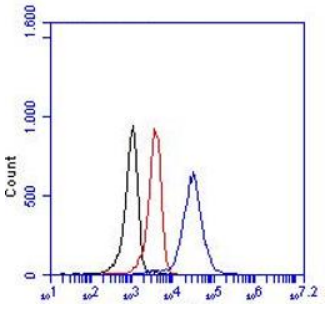
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	CD163, Human, clone RM3/1		
Catalog number	HM2157-100UG		
Lot number	-	**Expiry date**	-
Volume	1 ml	**Amount**	100 µg
Formulation	0.2 µm filtered in PBS+0.1%BSA	**Concentration**	100 µg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	<24 EU/mg	**Purification**	Protein G
Storage	4°C		

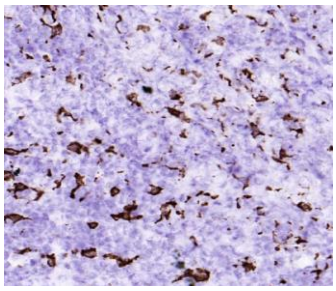
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	1		2	1-3	4		5	2,5
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



FC: Flow cytometric detection of CD163 in THP-1 cells. The black line represents cells only, the red line the isotype control and the blue line HM2157 in a concentration of 2 µg/250000 cells.



IHC-F: Staining of interfollicular tissue in frozen tonsil sections.

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 1:10.

- FC: Permeabilization as well as no permeabilization is used. Block cells with 1% BSA for 30'at 4°C (Ref 5)
- FS: Reduction of CD69 expression by adding 2.5 µg/ml RM3/1 to 5x105 human T-lymfocytes in presence of exogenous CD163 (Ref 2).
- IF: Transfected CHO-cells were plated on coverslips and fixed in 4% paraformaldehyde for 20'. Cells were stained with 4 µg/ml RM3/1 for 60'.
- IP: 10 µg RM3/1 was covalently coupled to beads (with 20 mM dimethyl pimelimidate), blocked with 0.2 M glycine buffer for 2 hr and incubated with cell lysate for 2 hr (Ref 5).
- W: SDS-PAGE under non-denatured condition. Block blot with 1% skimmed milk for 1h and incubate with RM3/1 (0.4 µg/ml) in 0.1%BSA/TBS for 1h. Detection of ~130 kDa product in spleens extracted in presence of NP-40 or in glucocorticoid-treated human monocytes. (Ref 2,5)
- Positive control: CHO-cells transfected with CD163; Negative control: Muscle cells.

General Information

Description

The monoclonal antibody RM3/1 recognizes CD163, a 130 kDa type I membrane glycoprotein, which is expressed exclusively in the monocyte/macrophage system. CD163 is a member of the cysteine-rich scavenger receptor superfamily. CD163 is an acute phase regulated receptor for the hemoglobin-haptoglobin complex. Another important function of CD163 seems to be in the adhesion of monocytes to activated endothelial cells. The expression levels of CD163 vary during the course of macrophage differentiation. The highest levels are found on tissue macrophages whereas bone marrow-derived cells are CD163 negative. CD163 positive cells include skin histiocytes, gut, Kupffer

cells, and macrophages in spleen, thymus placenta and inflamed or tumorous tissues. The protein expression is markedly induced by glucocorticoids, IL-6 and IL-10 while down-regulated by cyclosporin A and by phorbol esters. CD163 can be cleaved to release to soluble form (sCD163). PMA can induce the shedding of sCD163. Intravenous lipopolysaccharide (LPS) produces a rapid rise of sCD163 in plasma of patient as it induces metalloproteinase-mediated shedding from monocytes surface. sCD163 in plasma is a parameter in diseases affecting macrophage function and monocyte/macrophage load in the body. The concentration of sCD163 is probably reflecting the number of macrophages of the 'alternative macrophage activation' phenotype with a high CD163 expression playing a major role in dampening the inflammatory response and scavenging components of damaged cells. As such sCD163 can be important as a disease marker in inflammatory conditions e.g. infection, autoimmune disease, transplantation, atherosclerosis and cancer. The monoclonal antibody RM3/1 can be used for macrophage phenotyping..The RM3/1 antigen expression is restricted to human monocytes and macrophages that evolve in the late phase of inflammation, predominantly macrophages of healing tissue. For macrophages in the synovialis of patients with rheumatoid arthritis; in alveolar macrophages and in Kupffer cells a double staining can be observed with the monoclonal antibody 25F9 (HM2158) recognizing mature macrophages, which is not the case in other tissues.

Immunogen Human monocytes (epitope recognized SRCR domain 9)

Aliases Scavenger receptor cysteine-rich type 1 protein M130, Hemoglobin scavenger receptor, Intermediate Stage Inflammatory Macrophages.

Cross reactivity Monkey: Yes; Rat: No; Pig: No; Guinea pig: No.

References

1. Zwadlo, G et al; A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. Expl Cell Biol 1987, *55*: 295
2. Högger, P et al; Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine-rich family (CD163). J Immunol 1998, *161*: 1883
3. Droste, A et al; Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. Biochem Biophys Res Commun 1999, *256*: 110
4. Frings, W et al; Only the soluble form of the scavenger receptor CD163 acts inhibitory on phorbol ester-activated T-lymphocytes, whereas membrane-bound protein has no effect. FEBS Lett 2002, *526*: 93
5. Komohara, Y et al; AM-3K, an anti-macrophage antibody, recognizes CD163, a molecule associated with an anti-inflammatory macrophage phenotype. J Histochem Cytochem 2006, *54*: 763

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

Precautions For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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Approved by Manager of QC	Date
Brenda Teunissen	03/12/2019

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