GENTAUR

DATA SHEET

Monoclonal Antibody to CD163 - Azide Free

Alternate names:	Hemoglobin scavenger receptor, M130, Macrophage marker, Scavenger receptor cysteine-rich type 1 protein M130
Catalog No.:	AM20201AF-N
Quantity:	0.1 mg
Concentration:	1.0 mg/ml
Background:	CD163, also known as M130, Ber-Mac3, Ki-M8 or SM4, is a 130-150 kDa member of the scavenger receptor cysteine-rich (SRCR) superfamily. CD163 scavenges hemoglobin by mediating endocytosis of haptoglobin-hemoglobin complexes. CD163 expression is restricted to cells of monocyte lineage and increases as monocytes mature into macrophages. CD163 is up-regulated on mononuclear phagocytes by IL-10, IL-6, and dexamethasone, while lipopolysaccharide (LPS) and phorbol myristate acetate (PMA) induce shedding of CD163 from the cell surface. Several CD163 isoforms exist, which differ in their cytoplasmic domains and putative phosphorylation sites.
Uniprot ID:	Q86VB7
NCBI:	NP_004235
GeneID:	9332
Host / Isotype:	Mouse / IgG1
Clone:	Ber-Mac3
Immunogen:	Mononuclear splenocytes isolated from a normal donor after a traumatic rupture.
Format:	**State:** Liquid purified IgG fraction. **Purification:** Protein-A Agarose Chromatography of hybridoma supernatant. **Buffer System:** PBS, pH 7.2 containing 50% Glycerol without preservatives.
Applications:	**Flow Cytometry:** 10 µg/mL (final concentration). *Positive Control:* Monocyte. Detailed procedure is provided in **Protocols.** Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with CD163 antigen on Flow Cytometry.
Species Reactivity:	**Tested:** Human. **Expected from sequence similarity:** Monkey.
Add. Information:	This product was originally produced by MBL International.
Storage:	Store the antibody (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.

For research and in vitro use only. Not for diagnostic or therapeutic work.

General References:

1) Moniuszko, M., et al., Clin. Vaccine Immunol. 13, 704-707 (2006)
2) Pulford, K., et al., Immunology 75, 588-595 (1992)
3) Backé, E., et al., J. Clin. Pathol. 44, 936-945 (1991)
Clone Ber-Mac3 is used in these references.

Protocols:

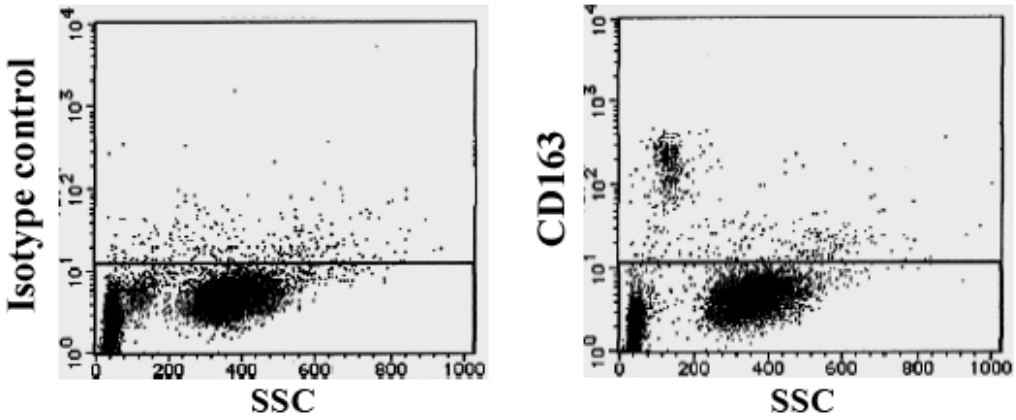
Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Wash the cells 3 times with washing buffer [PBS with 2% FCS and 0.1% NaN].
2) Resuspend the cells with washing buffer (5x10e6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at RT (20~25°C). Remove supernatant by careful aspiration.
4) Add 20 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at RT.
5) Add 40 µL of the primary antibody at the concentration of as suggest in the APPLICATIONS diluted in the washing buffer. Mix well and incubate for 30 minutes at RT.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at RT. Remove supernatant by careful aspiration.
7) Add 30 µL of 1:40 FITC conjugated anti-Mouse IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at RT.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at RT. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.
Positive Control: Monocyte

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all step described below.
1) Add 50 µL of the CD163 antibody (Clone: Ber-Mac3) as suggest in the APPLICATIONS diluted with the washing buffer [PBS with 2% FCS and 0.1% NaN3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at RT (20~25°C).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at RT. Remove supernatant by careful aspiration.
4) Add 30 µL of 1:40 FITC conjugated anti-mouse IgG diluted with washing buffer. Mix well and incubate for 15 minutes at RT.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
7) Add 1 mL of H2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Pictures:



Flow Cytometry: Analysis of CD163 expression on Human peripheral blood Leoukocytes.

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