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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-CD93 (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
D198-5	mNI-11	Mouse IgG1	1 mL (50 tests)

BACKGROUND: CD93, also known as C1qRp, is a 100-125 kDa type I membrane protein that is a receptor for the complement protein C1q. CD93 has also been shown to act as a receptor for mannose-binding lectin and surfactant protein A. CD93 is expressed on the surface of monocytes, granulocytes, and endothelial cells, and expression is increased by TNF-α or GM-CSF. CD93 is involved in ligand-mediated enhancement of phagocytosis and intercellular adhesion.

SOURCE: This antibody was purified from hybridoma (clone mNI-11) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with LPS-stimulated U937.

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN_3.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with CD93 antigen on Flow cytometry.

APPLICATIONS:
Flow cytometry; 20 µL (ready for use)
*Please refer to the data sheet (MBL; code no. D198-3) for other applications.

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

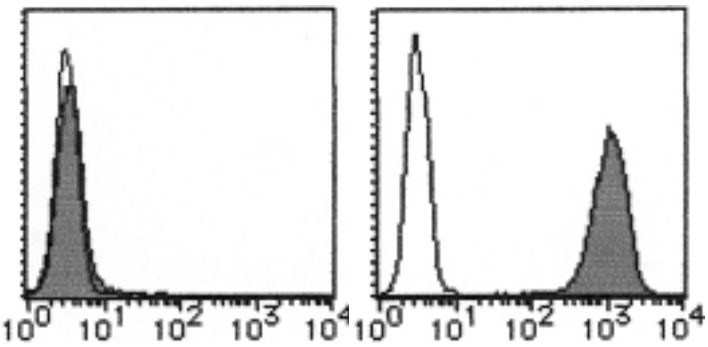
REFERENCES:
1) Ikewaki, N., *et al*., *Microbiol. Immunol.* **57**, 822-832 (2013)
2) Ikewaki, N., *et al*., *J. Clin. Immunol.* **30**, 723-733 (2010)
3) Ikewaki, N., *et al*., *Microbiol. Immunol.* **51**, 1189-1200 (2007)
4) Ikewaki, N., *et al*., *Microbiol. Immunol.* **50**, 93-103 (2006)
5) Ikewaki, N., *et al., J. of Kyushu Univ. of Health and Welfare* **7**, 183-189 (2006)
6) McGreal, E. P., Ikewaki, N., *et al., J. Immunol*. **168**, 5222-5232 (2002)
7) Ikewaki, N., *et al., J. Clin. Immunol*. **20**, 317-324 (2000)
8) Ikewaki, N., *et al., J. Leukoc. Biol*. **59**, 697-708 (1996)

Clone mNI-11 is used in these references.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	U937, Monocyte, Granulocyte, Naive T lymphocytes ($CD4^+$ $CD45RA^+$ cells) *	Not Tested	Not Tested
Reactivity on FCM	+		

*It is reported that clone mNI-11 reacted with naive T lymphocyte in neonatal umbilical cord blood in the reference number 2).



Flow cytometric analysis of CD93 expression on Jurkat cells (left) and U937 cells (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D198-5 to the cells.*

PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).

3) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 20 μL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add the primary antibody as suggested in the **APPLICATIONS**. Mix well and incubate for 20 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; U937)

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

1) Add tthe primary antibody as suggested in the **APPLICATIONS** into each tube.
2) Add 50 μL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) 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.
5) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

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