For Research Use Only.
Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-CD93 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D198-3	mNI-11	Mouse IgG1	100 µL	1 mg/mL

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: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with CD93 antigen on Immunoprecipitation, Immunohistochemistry and Flow cytometry. mNI-11 has been reported to induce intercellular adhesion of LPS-stimulated monocyte (U937) cells as well as inducing the adhesion of these cells to an endothelial (HUVEC) cell layer and rapid spread formation in HUVECs.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 2 µg/400 µg of cell extract from $5x10^6$ cells
Immunohistochemistry; 10 µg/mL
This antibody is available for the frozen section.
Immunocytochemistry; Not tested
Flow cytometry; 5 µg/mL (final concentration)

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

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

SPECIES CROSS REACTIVITY:

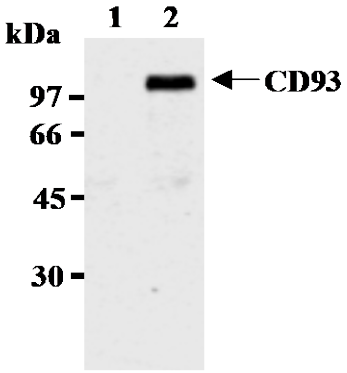
Species	Human	Mouse	Rat
Cells	U937, Monocyte, Granulocyte, Naive T lymphocytes ($CD4^+$ $CD45RA^+$ cells) *	Not tested	Not tested
Reactivity on FCM	+		

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

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., *et al., J. Immunol.* **168**, 5222-5232 (2002)
7) Ikewaki, N., *et al., J. Clin. Immunol.* **20**, 317-324 (2000)
8) Ikewaki, N., and Inoko, H., *J. Leukoc. Biol.* **59**, 697-708 (1996)

Clone mNI-11 is used in these references.



Immunoprecipitation of CD93 from U937 cells with Mouse IgG1 (1) or D198-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP-Streptavidin.

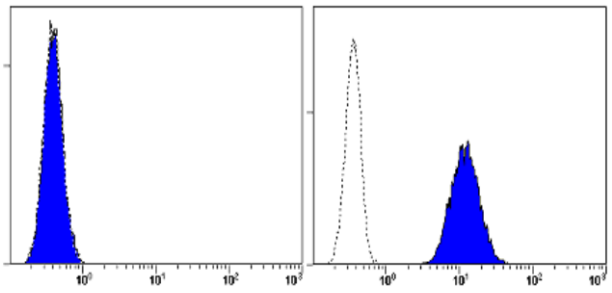
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Immunoprecipitation

1) Wash the biotin labeled U937 cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 400 µg of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
8) Incubate the membrane with the 1:10,000 HRP-conjugated streptavidin (MBL; code no. IM-0309) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS (5 minutes x 6 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; U937)



Flow cytometric analysis of CD93 expression on U937 cells (right) and Jurkat cells (left). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D198-3 to the cells.*

Flow cytometric analysis for floating cells

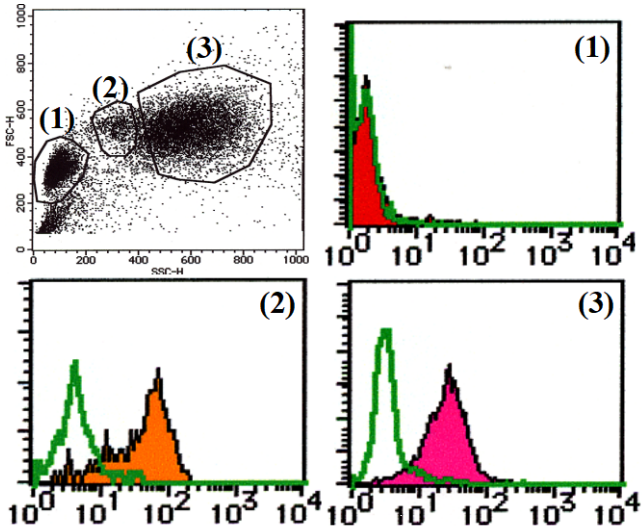
We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

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 (5x10^6 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 40 µL of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 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) Add 20 µL of 1:100 anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) 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.
9) 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



Flow cytometric analysis of CD93 expression on lymphocytes (1), monocytes (2) and granulocytes (3). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D198-3 to the cells.*

for all steps described below.

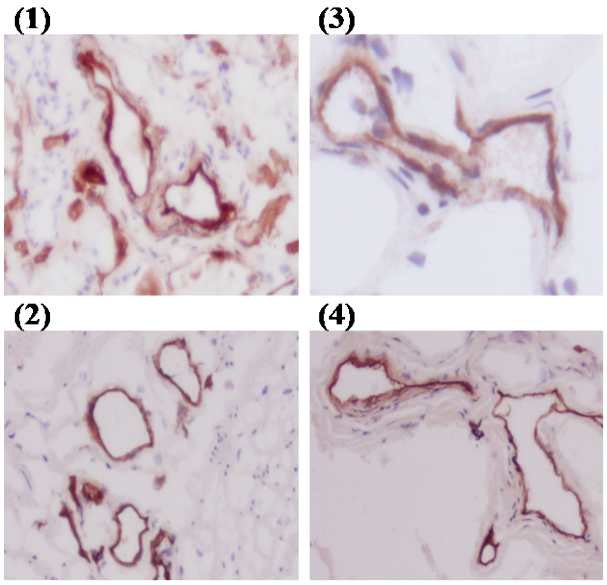
1) Add 50 µL of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (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) Add 30 µL of 1:100 anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
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 H_2O 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.

(Positive controls for Flow cytometry; monocytes, granulocytes)

Immunohistochemical staining for frozen sections

1) Fix the sections with cold acetone for 5 minutes.
2) After the slides dry up, immerse the slides into PBS.
3) Immerse the slides into 5% H_2O_2 for 10 minutes at room temperature to block the endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
4) Remove the slides from PBS, wipe gently around each section and cover tissues with 5% skimmed milk in PBS for 30 minutes at 37°C to block non-specific staining. Wash 3 times in PBS for 5 minutes each.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with the CD93 monoclonal antibody (mNI-11) diluted with PBS as suggest in the **APPLICATIONS**.
6) Incubate the sections for 1 hour at 37°C.
7) Wash the slides 3 times in PBS for 5 minutes each.
8) Wipe gently around each section and cover tissues with HRP-conjugated dextran-70 bound anti-mouse Ig (ENVISION Kit/HRP, DaKo; code no. K5027). Incubate for 40 minutes at room temperature. Wash as in step 7).
9) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
10) Wash the slides in water for 5 minutes.
11) Counter stain in hematoxylin for 1-5 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
12) Now ready for mounting.

(Positive control for Immunohistochemistry; human vascular endothelial cells)



Immunohistochemical detection CD93 on frozen section of vascular endothelial cells in human kidney (1), human colon (2), human testis (3) and human bladder (4) with D198-3.

These frozen sections were kindly provided by Dr. Hiroaki Kataoka, M.D. (Second Department of Pathology, Miyazaki University School of Medicine) and Dr. Nobunao Ikewaki, Ph.D. (Institute of Immunology, Kyushu University of Health and Welfare).

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.