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

MONOCLONAL ANTIBODY

Anti-CD73 (Mouse)

Code No.	Clone	Subclass	Quantity	Concentration
D294-3	23-9	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: CD73 (NT5E, ecto 5' nucleotidase, E5NT, NTE) is a 70 kDa of glycosyl phosphatidylinositol (GPI) linked molecule which can be detected in several different mammalian tissues and cell types. CD73 enzyme activity catalyzes the extracellular nucleoside monophosphates into bioactive nucleoside intermediates. This enables the uptake of adenosine, inosine, and guanosine into the cell and their subsequent reconversion into ATP and GTP in the purine salvage pathway. CD73 expressed on B cell subset, T cell subset and vascular endothelial cells. Subsets of T and B lymphocytes are CD73 positive and the level of expression increases with lymphocyte maturation.

SOURCE: This antibody was purified from hybridoma (clone 23-9) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with Wistar rat lymphocyte immunized with mouse fetal hepatic cells.

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 mouse CD73 on Flow cytometry.

APPLICATION:
Flow cytometry; 5 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	Transfectant	Not Tested
Reactivity on FCM		+	

INTENDED USE:
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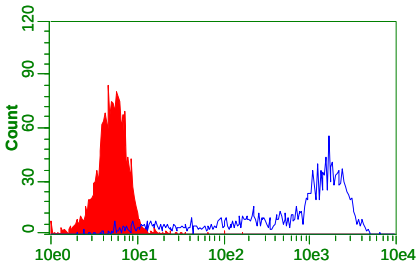
REFERENCES:
1) Kakinuma, S., *et al.*, *J. Hepatol.* **51**, 127-138 (2009)
2) Resta, R., *et al.*, *Immunol. Rev.* **161**, 95-109 (1998)



Flow cytometric detection of CD73 (Mouse) on Ba/F3 transfectant
Open: D294-3
Closed: isotype control

PROTOCOL:
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)].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 100 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 10 µL of normal goat serum to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 µL of the primary antibody at the concentration as suggested in the **APPLICATION** 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 40 µL of 1:50 PE conjugated anti-rat IgG (MBL; code no. IM-1623) 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; transfectant)

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RELATED PRODUCTS:

M081-3	Rat IgG2a isotype control (2H3)
IM-1623	Anti-rat IgG-PE (polyclonal)