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MONOCLONAL ANTIBODY

Anti-CD63 (LAMP-3) (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D263-3	R5G2	Rat IgG2b	100 µL	1 mg/mL

BACKGROUND: CD63 is not only expressed on activated platelets, but also activated monocytes and macrophages, and is weakly expressed on granulocytes, T cell and B cells. It is located on the basophilic granule membranes and translocated to cell surface upon various stimuli. The membrane of lytic granules in CTLs contains CD63/LAMP-3 and other lysosomal-associated glycoproteins (LAMPs) such as CD107a/LAMP-1 and CD107b/LAMP-2. LAMPs have been observed on the cell surface as a result of degranulation. CD63 belongs to a member of the tetraspanin transmembrane-protein (TM4) superfamily, which includes CD9, CD37, CD53, CD81, CD82, CD151 and CD231. Several members of this family form noncovalent associations with integrins, particularly β1 integrins (CD29), and modulate cellular adhesion properties. CD63 has a tyrosine-based internalization motif in the cytoplasmic C-terminal tail and interacts with adaptor protein complexes such as AP-2 and AP-3. Because AP-2 and AP-3 are involved in facilitating the clathrin-mediated endocytosis, CD63 could be directly involved in the internalization of its membrane protein partners.

SOURCE: This antibody was purified from hybridoma (clone R5G2) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Sprague-Dawley rat splenocyte immunized with mouse bone marrow stroma cell line (ST2).

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 CD63 on Western blotting and Flow cytometry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Transfectant	WEHI-3B	Not tested
Reactivity on FCM	-	+	

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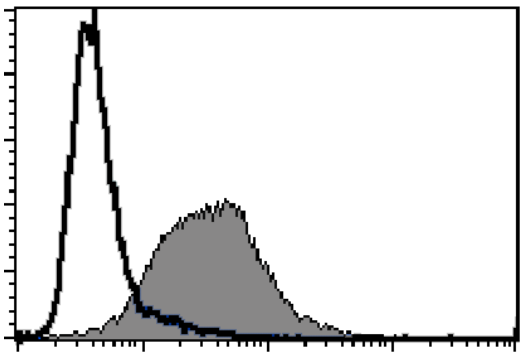
APPLICATIONS:
Western blotting; 2-10 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in Immunocytochemistry in the reference number 2)-4), 6) and 7).
Flow cytometry; 10 µg/mL (final concentration)

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

REFERENCES:
1) Zonneveld, M. I., *et al*., *J. Extracell. Vesicles* **3**, 24215 (2014) [WB]
2) Bobrie, A., *et al., J. Extracell. Vesicles* **1**, 18397 (2012) [WB, IC]
3) Lopes da Silva, M., *et al., Traffic* **13**, 1351-1363 (2012) [IC]
4) Päll, T., *et al*., *PLoS One* **6**, e29305 (2011) [IC]
5) Verjan Garcia, N., *et al., J. Immunol.* **187**, 2268-2277 (2011) [FCM]
6) Ushio, H., *et al*., *J. Allergy Clin. Immunol.* **127**, 1267-1276 (2011) [WB, IC, FCM]
7) Seto, S., *et al*., *Microbiol. Immunol.* **54**, 170-174 (2010) [WB, IC]
8) Kunert, S., *et al., Blood* **114**, 5532-5540 (2009) [FCM]
9) Liu, Y., *et al., J. Exp. Med*. **204**, 93-103 (2007)
10) Kabu, K., *et al., J. Immunol*. **177**, 1296-1305 (2006)
11) Nishida, K., *et al., J. Cell Biol*. **170**, 115-126 (2005)
12) Mitsunari, T., *et al., Mol. Cell Biol*. **25**, 9318-9323 (2005)

Clone R5G2 is used in these references.



Flow cytometric analysis of mouse CD63 expression on WEHI-3B. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of anti-mouse CD63 (clone R5G2, MBL, code no. D263-3) to the cells.*

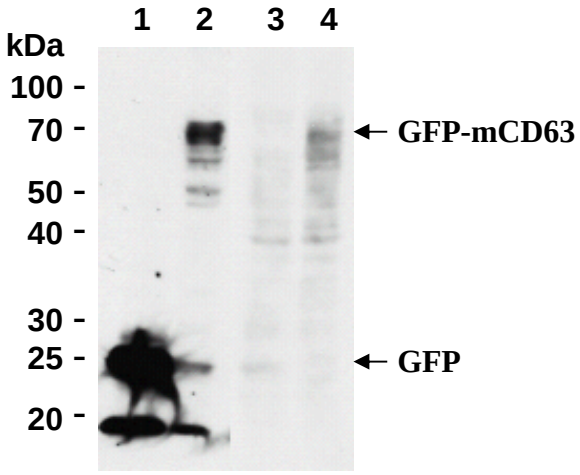
PROTOCOLS:

Flow cytometric analysis for floating cells

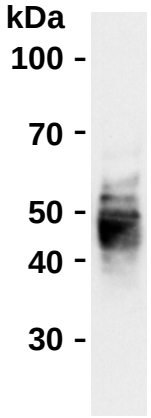
We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

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 10 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 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 30 µL of 1:100 Anti-IgG (Fc) (Rat) pAb-PE (Beckman Coulter, code no. IM0552) 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; WEHI-3B)



Western blotting analysis of mouse CD63 expression in GFP-tagged mouse CD63 transfected 293T (2, 4) and GFP transfected 293T (1, 3) using anti-GFP antibody (clone 1E4, lane 1 and 2, MBL, code no. M048-3) or anti-mouse CD63 (clone R5G2, lane 3 and 4, MBL, code no. D263-3).



Western blotting analysis of mouse CD63 expression in BMMCs (mouse bone marrow-derived mast cells) using anti-mouse CD63 (clone R5G2, MBL, code no. D263-3).

SDS-PAGE & Western blotting

1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 5 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) 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% methanol). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 to 3 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with the 1:10,000 Anti-IgG (Rat) pAb-HRP (Beckman Coulter, code no. IM0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (10 minutes x 3).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
10) 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 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; mouse bone marrow-derived mast cells)

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