

Product datasheet for **AM26556AF-N**

Cd63 Rat Monoclonal Antibody [Clone ID: R5G2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	R5G2
Applications:	FC, WB
Recommended Dilution:	Western blot: 2-10 µg/ml for chemiluminescence detection system. Flow cytometry: 10 µg/ml (final concentration). For details see protocols below.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Mouse bone marrow stroma cell line (ST2)
Specificity:	This antibody reacts with mouse CD63.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein G agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	CD63 antigen
Database Link:	Entrez Gene 12512 Mouse P41731



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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 β 1 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.

Synonyms:

OMA81H, Granulophysin, Tetraspanin-30, MLA1, TSPAN30, ME491

Note:

This product was originally produced by MBL International.

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) and 0.1% NaN₃].
- 2) Resuspend the cells with washing buffer (5×10^6 cells/mL).
- 3) Add 50 μ L 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 μ L 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 room temperature.
- 5) Add 40 μ L of the primary antibody at the concentration as suggest 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 μ L of 1:100 PE conjugated anti-rat IgG 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 μ L of the washing buffer and analyze by a flow cytometer. (Positive control for Flow cytometry; WEHI-3B)

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 μ L 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/cm² 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.

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 PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 to 3 hours 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 times).

7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rat IgG 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 times).

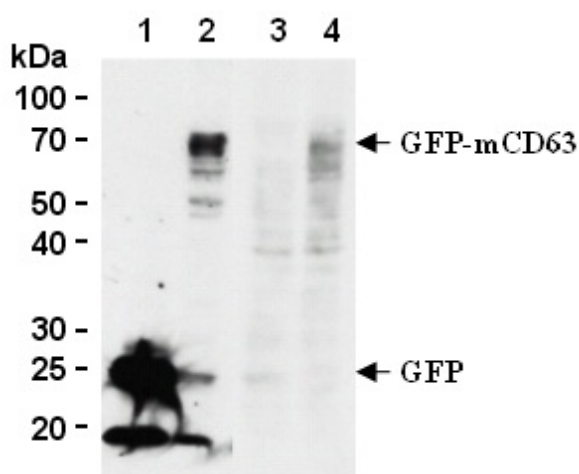
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.

10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.

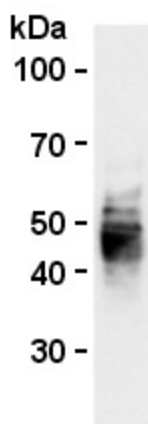
11) Expose to an X-ray film in a dark room for 3 minutes.

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)

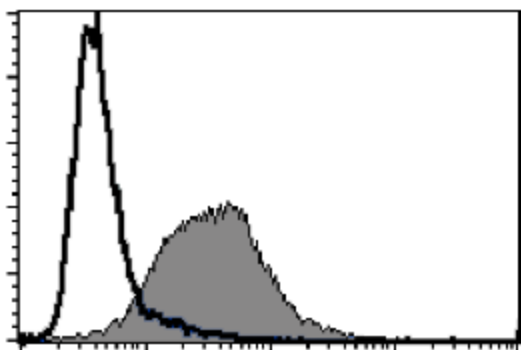
Product images:



Western blot 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) or anti-mouse CD63 (clone R5G2, lane 3 and 4, AM26556AF-N).



Western blot analysis of mouse CD63 expression in BMMCs (mouse bone marrow-derived mast cells) using anti-mouse CD63 (clone R5G2, AM26556AF-N).



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, code no. AM26556AF-N) to the cells.