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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 aliqouts) 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

View online »

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 b een 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 ß 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

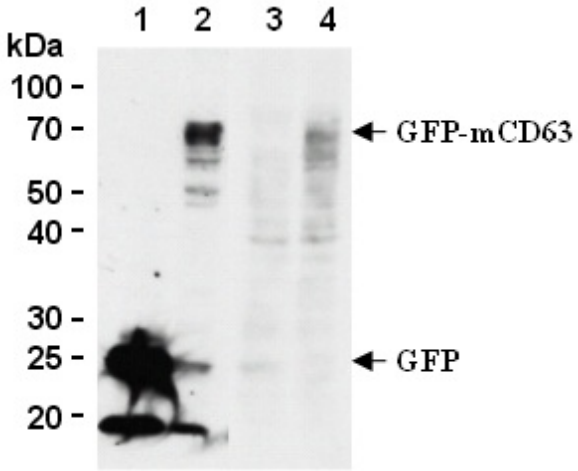
We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN 3 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 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 minut es 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

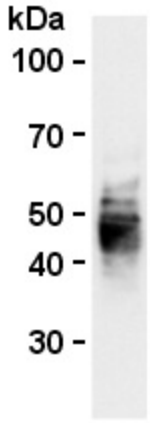
1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 5 minutes and centrifuge. Load 20 µ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm 2 for 1 hour in

a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufact ure'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 suggest 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 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)

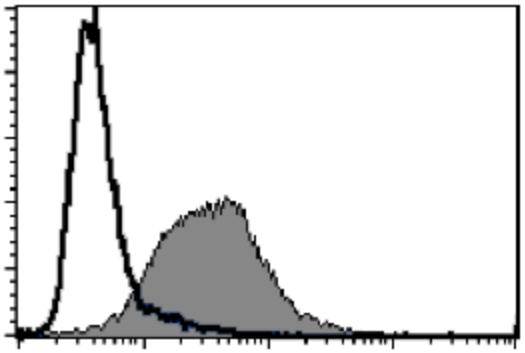
Product images:



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.