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

Anti-CD63 (LAMP-3) mAb-Biotin

CODE No.	MEX002-6
CLONALITY	Monoclonal
CLONE	C047-1
ISOTYPE	Mouse IgG2b κ
QUANTITY	50 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Human prostate carcinoma cell line (PC3) derived exosomes (prepared by ultracentrifugation from cultured supernatant)
FORMULATION	PBS containing 1% BSA and 0.1% ProClin 950
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATIONS-CONFIRMED

Western blotting	1 µg/mL
Flow cytometry	5 µg/mL
Sandwich CLEIA	Can be used.
Exosome isolation	Can be used.

APPLICATION-UNDER EVALUATION

Sandwich ELISA	Can be used.

SPECIES CROSS REACTIVITY on WB

Species	Human	Monkey	Mouse	Rat	Hamster
Cells	HeLa, HEK293T	Not tested	WR19L	Not tested	Not tested
Reactivity	+		-		

Entrez Gene ID 967 (Human)

REFERENCES

1) Melo, S. A., *et al., Nature* **523**, 177-182 (2015)
2) Yoshioka, Y., *et al., Nat. Commun.* **5**, 3591 (2014)
3) Pols, M. S. and Klumperman, J., *Exp. Cell Res.* **315**, 1584-1592 (2009)
4) Simons, M. and Raposo, G., *Curr. Opin. Cell Biol.* **21**, 575-581 (2009)
5) Liu, Y., *et al., J. Exp. Med.* **204,** 93-103 (2007)
6) Kabu, K., *et al., J. Immunol.* **177,** 1296-1305 (2006)
7) Nishida, K., *et al., J. Cell Biol.* **170,** 115-126 (2005)
8) Mitsunari, T., *et al., Mol. Cell. Biol.* **25,** 9318-9323 (2005)

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MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
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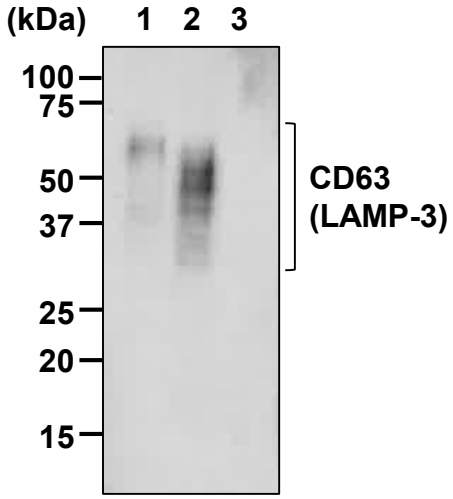
URL https://ruo.mbl.co.jp e-mail support@mbl.co.jp

SDS-PAGE & Western blotting (non-reducing condition)

1) Wash 1 × 10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer (non-reducing condition), then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 10 V for 50 min. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. × 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. × 3 times).
8) Incubate the membrane with the 1:20,000 Streptavidine-Horseradish Peroxidase (GE Healthcare; code no. RPN4401) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. × 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. 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 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa and HEK293T)



Western blotting analysis of CD63 (LAMP-3) protein

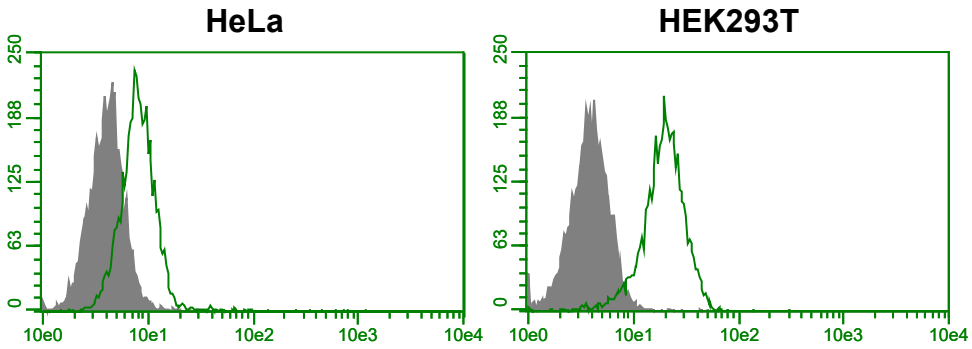
Lane 1: HeLa
Lane 2: HEK293T
Lane 3: WR19L

Immunoblotted with Anti-CD63 (LAMP-3) mAb-Biotin (MEX002-6)

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) 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 10 min. at room temperature.
3) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with washing buffer.
4) Mix well and incubate for 20 min. at room temperature.
5) Wash the cells 1 time with 1 mL of washing buffer.
6) Add FITC-conjugated streptavidin diluted with washing buffer. Mix well and incubate for 20 min. at room temperature.
7) Wash the cells 1 time with 1 mL of washing buffer.
8) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

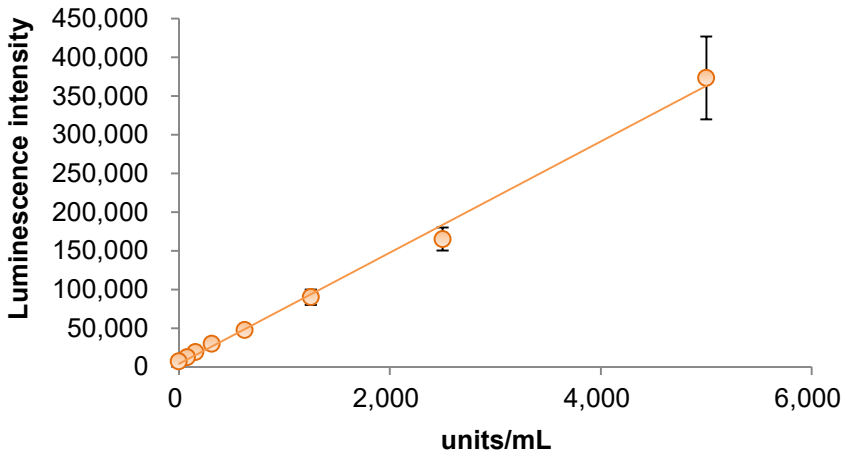
(Positive controls for Flow cytometry; HeLa and HEK293T)



Flow cytometric detection of human CD63 (LAMP-3)
Left: HeLa
Right: HEK293T

Open: Anti-CD63 (LAMP-3) mAb-Biotin (MEX002-6)
Closed: Mouse IgG2b (isotype control)-Biotin (M077-6)

Sandwich CLEIA (chemiluminescence enzyme immunoassay)



Sandwich CLEIA for measurement of CD63 (LAMP-3) expressed HeLa-derived exosomes
Sample: HeLa-derived exosomes prepared by ultracentrifugation
Capture Antibody: Anti-CD81 (TAPA1) mAb (MEX003-3)
Detection Antibody: Anti-CD63 (LAMP-3) mAb-Biotin (MEX002-6)