

# Anti-CD63 (LAMP-3) mAb-Biotin

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

## APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1 $\mu$ g/mL
<u>Flow cytometry</u>	5 $\mu$ g/mL
<u>Sandwich CLEIA</u>	Can be used.
<u>Exosome isolation</u>	Can be used.

## APPLICATION-UNDER EVALUATION

<u>Sandwich ELISA</u>	Can be used.
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## 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)

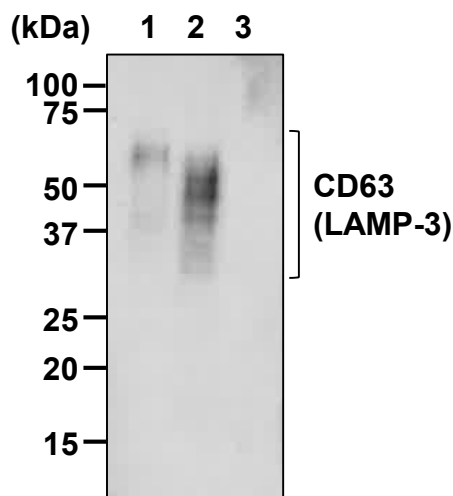
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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

- 1) Wash  $1 \times 10^7$  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  $\mu$ L 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.  $\times$  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.  $\times$  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.  $\times$  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***

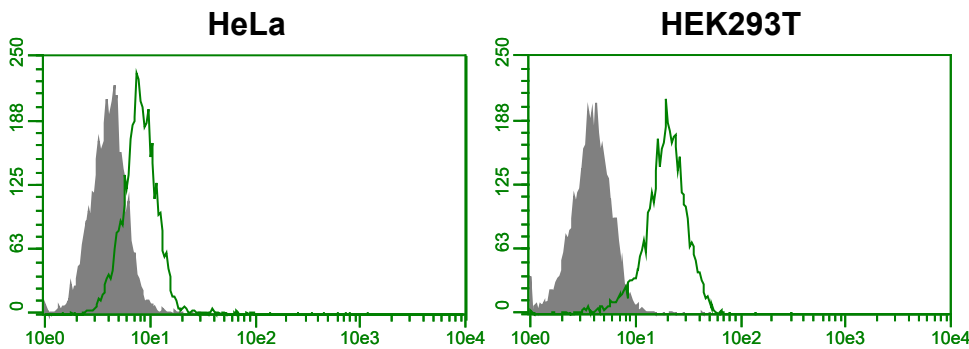
Lane 1: HeLa  
Lane 2: HEK293T  
Lane 3: WR19L

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

### **Flow cytometric analysis**

- 1) Wash the cells ( $5 \times 10^5$  cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Add 10  $\mu$ L 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  $\mu$ L 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  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; HeLa and HEK293T)

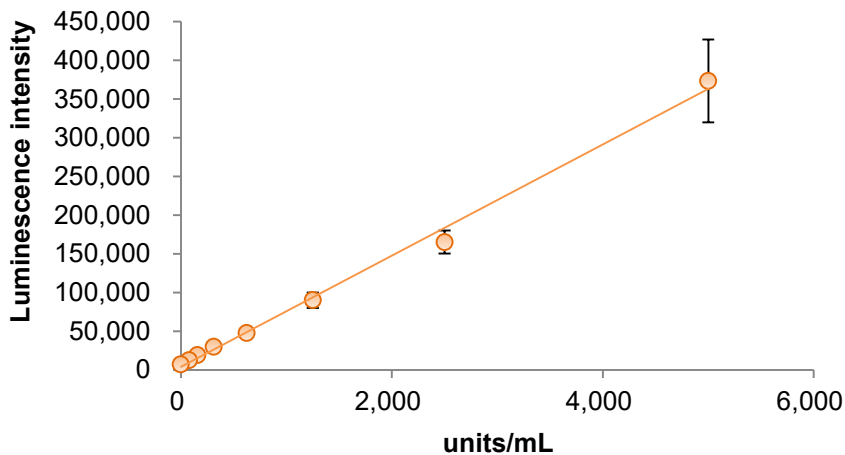


### ***Flow cytometric detection of human CD63 (LAMP-3)***

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)