

Anti-CD63 (LAMP-3) mAb

CODE No.	MEX002-3
CLONALITY	Monoclonal
CLONE	C047-1
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 μ L, 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 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.

APPLICATIONS

<u>Western blotting</u>	1 μ g/mL
<u>Immunoprecipitation</u>	5 μ g/sample
<u>Flow cytometry</u>	5 μ g/mL

APPLICATIONS-UNDER EVALUATION

<u>Sandwich ELISA</u>	Can be used.
<u>Sandwich CLEIA</u>	Can be used.

SPECIES CROSS REACTIVITY on WB

Species	Human*	Monkey	Mouse	Rat	Hamster
Cells	HeLa-derived exosomes, HeLa, HEK293T, K562	COS-7	NIH/3T3, WR19L	Rat1	CHO-K1
Reactivity	+	+	-	-	-

*This antibody does not react with Jurkat cells.

Entrez Gene ID 967 (Human), 103238483 (Monkey)

REFERENCES

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- 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)

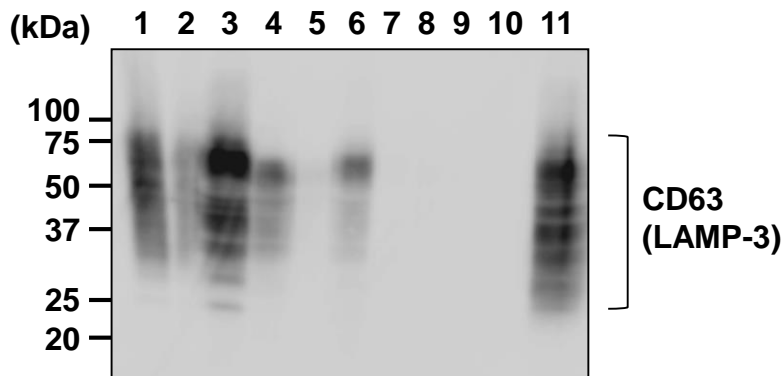
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SDS-PAGE & Western blotting (non-reducing condition)

- 1) Wash 1×10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer (non-reducing condition), and then sonicate briefly (up to 10 sec.) .
- 2) Boil the samples for 5 min. and centrifuge. Load 10 μ 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 2 mA/cm² for 70 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) for 1 hr. at room temperature.
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. \times 2).
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** overnight at 4°C. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (5 min. \times 3).
- 8) Incubate the membrane with the 1:5,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 30 min. at room temperature.
- 9) Wash the membrane with PBS-T (5 min. \times 3) .
- 10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 30 sec. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose for 20 sec. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure may vary.

(Positive controls for Western blotting; HeLa-derived exosomes, HeLa, HEK293T, K562 and COS-7)



Western blotting analysis of CD63 (LAMP-3) protein

- Lane 1: HeLa-derived exosomal lysate (1 μ g)
- Lane 2: HeLa-derived exosomal lysate (0.3 μ g)
- Lane 3: HeLa
- Lane 4: HEK293T
- Lane 5: Jurkat
- Lane 6: K562
- Lane 7: NIH/3T3
- Lane 8: WR19L
- Lane 9: Rat1
- Lane 10: CHO-K1
- Lane 11: COS-7

Immunoblotted with Anti-CD63 (LAMP-3) mAb (MBL, code no. MEX002-3)

Immunoprecipitation from cultured cells

[Material Preparation]

1. Lysis Buffer (+) (MBL; code no. RN1001)

Before using the Lysis Buffer, protease inhibitors are added to the Lysis Buffer at the appropriate concentration.

2. Antibody conjugated Protein G-Magnetic Beads

A) Wash 75 μ L of Protein G-Magnetic Beads (MBL; code no. MJS002V2) slurry 1 time with 1 mL of Wash Buffer (MBL; code no. RN1001). Place the tube on Magnetic Rack (MBL; code no. 3190) for about 1 min. to separate the beads from the solution, and then carefully discard the supernatant.

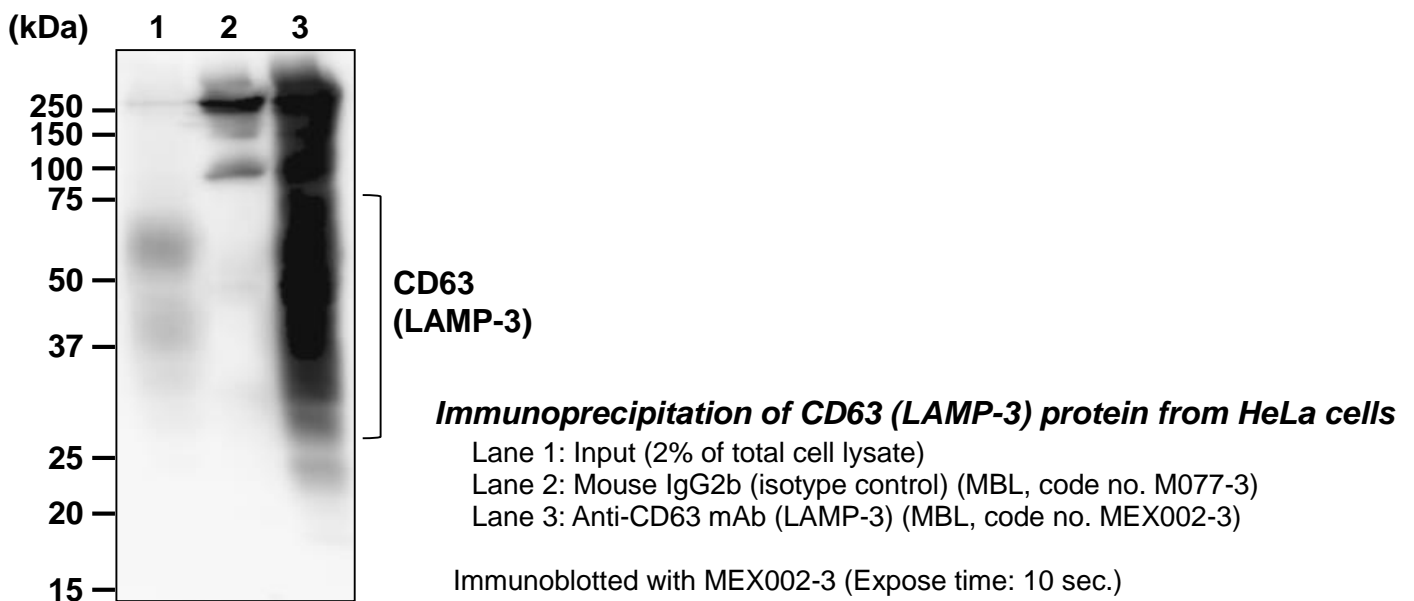
B) Mix the beads slurry resuspended in 1 mL of Wash Buffer with Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-CD63 mAb (MBL; code no. MEX002-3) at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation for 2 hr. at 4°C.

C) During pre-clear steps (Protocol 4)), wash the beads 1 time with 1 mL of Wash Buffer. Place the tube on Magnetic Rack for about 1 min., and then carefully discard the supernatant. Incubate at 4°C until just before use.

[Protocol]

- 1) Wash 5×10^6 cells 3 times with PBS and suspend them with 500 μ L of Lysis Buffer (+). Vortex thoroughly, then incubate on ice for 10 min.
- 2) Centrifuge the tube at $12,000 \times g$ for 10 min. at 4°C and transfer the supernatant to another tube.
- 3) Wash 75 μ L of Protein G-Magnetic Beads (MBL; code no. MJS002V2) 1 time with 1 mL of Wash Buffer (MBL; code no. RN1001). Place the tube on Magnetic Rack for about 1 min., and then carefully discard the supernatant.
- 4) [Pre-clear] Add the supernatant (prepared in step 2) to the tube containing the beads. Incubate it at 4°C with rotating for 1 hr.
- 5) Place the tube on Magnetic Rack (MBL; code no. 3190) for about 1 min., and then transfer the supernatant to the tube containing antibody conjugated Protein G-Magnetic Beads. Incubate it with gentle agitation overnight at 4°C.
- 6) Wash the beads 4 times with 1 mL of Wash Buffer. Place the tube on Magnetic Rack for about 1 min., and then carefully discard the supernatant.
- 7) Resuspend the beads in 25 μ L of Laemmli's sample buffer (non-reducing condition), boil for 5 min. and centrifuge.
- 8) Use 10 μ L/lane for the SDS-PAGE Analysis. (See **SDS-PAGE & Western blotting**)

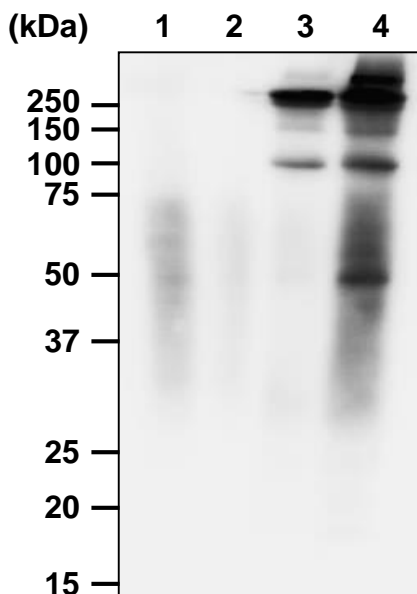
(Positive control for Immunoprecipitation; HeLa)



Immunoprecipitation from exosomes prepared by ultracentrifugation from cell culture supernatant

- 1) Prepare Antibody conjugated Protein G-Magnetic Beads:
 - A) Wash 75 μ L of Protein G-Magnetic Beads (MBL; code no. MJS002V2) slurry 1 time with 1 mL of PBS. Place the tube on Magnetic Rack (MBL; code no. 3190) for about 1 min. to separate the beads from the solution, and then carefully discard the supernatant.
 - B) Mix the beads slurry resuspended in 1 mL of PBS with Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-CD63 mAb (MBL; code no. MEX002-3) at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation for 2 hr. at 4°C.
 - C) During pre-clear steps (Protocol 3)), wash the beads 1 time with 1 mL of PBS. (Place the tube on Magnetic Rack for about 1 min., and then carefully discard the supernatant.) Incubate at 4°C until just before use.
- 2) Wash 75 μ L of Protein G-Magnetic Beads 1 time with 1 mL of PBS. Place the tube on Magnetic Rack for about 1 min., and then carefully discard the supernatant.
- 3) [Pre-clear] Add 30 μ g of exosomes resuspended in PBS. (final volume: 500 μ L) Incubate it at 4°C with rotating for 1 hr.
- 4) Place the tube on Magnetic Rack for about 1 min., and then transfer the supernatant to the tube containing antibody conjugated Protein G-Magnetic Beads. Incubate it with gentle agitation overnight at 4°C.
- 5) Wash the beads 4 times with 1 mL of PBS. Place the tube on Magnetic Rack for about 1 min., and then carefully discard the supernatant.
- 6) Resuspend the beads in 25 μ L of Laemmli's sample buffer (non-reducing condition), boil for 5 min. and centrifuge.
- 7) Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 2 mA/cm² for 1 hr. 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.
- 9) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 10) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. \times 2].
- 11) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** overnight at 4°C. (The concentration of antibody will depend on the conditions.)
- 12) Wash the membrane with PBS-T (5 min. \times 3).
- 13) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 30 min. at room temperature.
- 14) Wash the membrane with PBS-T (5 min. \times 3).
- 15) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 30 sec.
- 16) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 17) Expose for 10 sec. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure may vary.

(Positive control for Immunoprecipitation; HeLa-derived exosomes)

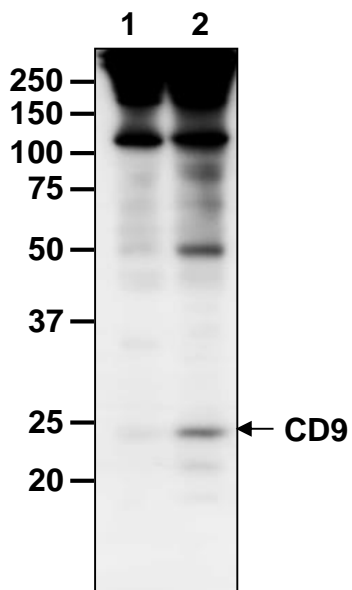


Immunoprecipitation of CD63 (LAMP-3)-expressed exosomes

- Sample: HeLa-derived exosomes
Lane 1: Input (1 mg of exosomes)
Lane 2: Input (0.3 mg of exosomes)
Lane 3: IP with Mouse IgG2b (isotype control) (MBL, code no. M077-3)
Lane 4: IP with Anti-CD63 (LAMP-3) mAb (MBL, code no. MEX002-3)

Immunoblotted with MEX002-3

Immunoprecipitation from human serum (under evaluation)



Immunoprecipitation of exosomes derived from human serum

Sample: 500 μ L of 2 x diluted pooled human serum

Lane 1: IP with Mouse IgG2b (isotype control) (MBL, code no. M077-3)

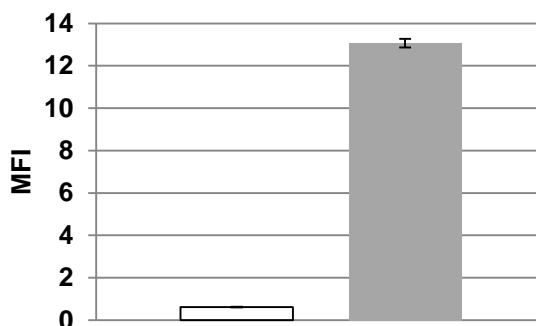
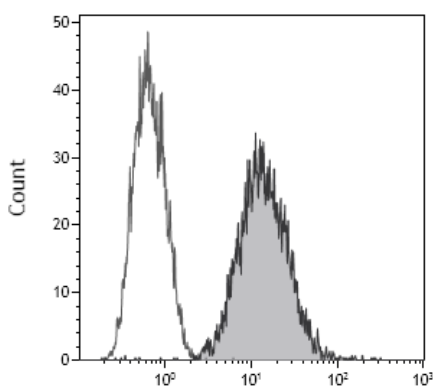
Lane 2: IP with Anti-CD63 (LAMP-3) mAb (MBL, code no. MEX002-3)

Immunoblotted with Anti-CD9 mAb (MBL, code no. MEX001-3)

Flow cytometric analysis

- 1) Detach the cells from the cell culture dish by treating with PBS containing 5 mM EDTA.
- 2) Wash the cells by resuspending the cell pellet with PBS.
- 3) Aliquot the cell suspensions into 96-well plate (1×10^5 cells/well).
- 4) Wash the cells 2 times with Wash buffer [PBS containing 0.5% BSA, 2 mM EDTA and 0.05% NaN_3].
- 5) Add 50 μL of Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-CD63 mAb (MBL; code no. MEX002-3) diluted with Wash buffer as suggested in the **APPLICATIONS**. Mix well and incubate for 1 hr. at room temperature.
- 6) Wash the cells 2 times with Wash buffer.
- 7) Add 50 μL of PE-conjugated anti-mouse IgG antibody diluted with Wash buffer. Mix well and incubate for 30 min. at room temperature.
- 8) Wash the cells 3 times with Wash buffer.
- 9) Resuspend the cells with 600 μL of Wash buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; HeLa)



Flow cytometric detection of human CD63 (LAMP-3)

Cells: HeLa

Open: Mouse IgG2b (isotype control) (MBL, code no. M077-3)

Closed: Anti-CD63 (LAMP-3) mAb (MBL, code no. MEX002-3)