

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



Anti-CD9 mAb-Biotin

CODE No.	MEX001-6
CLONALITY	Monoclonal
CLONE	A100-4
ISOTYPE	Mouse IgG2a κ
QUANTITY	50 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 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

<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system (<u>non-reducing condition</u>)
<u>Flow cytometry</u>	5 μ g/mL
<u>Sandwich CLEIA</u>	Can be used. Please refer to " <u>ExoDiluent for Immunoassay (MEX1001)</u> " together.
<u>Exosome isolation</u>	Can be used. Please refer to " <u>ExoCap™ Streptavidin Kit (MEX-SA)</u> " together.

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
Cell	HeLa, HEK293T	Not tested	WR19L	Not tested	Not tested
Reactivity	+		-		

Entrez Gene ID 928 (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) Boucheix, C., *et al.*, *J. Biol. Chem.* **266**, 117-122 (1991)

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MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL <http://ruo.mbl.co.jp/>
e-mail support@mbi.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

MEX001-3	Anti-CD9 mAb (A100-4)
MEX001-4	Anti-CD9 mAb-FITC (A100-4)
MEX001-6	Anti-CD9 mAb-Biotin (A100-4)
MEX001-12	Anti-CD9 mAb-ALP (A100-4)
MEX002-3	Anti-CD63 (LAMP-3) mAb (C047-1)
MEX002-4	Anti-CD63 (LAMP-3) mAb-FITC (C047-1)
MEX002-6	Anti-CD63 (LAMP-3) mAb-Biotin (C047-1)
MEX002-12	Anti-CD63 (LAMP-3) mAb-ALP (C047-1)
MEX003-3	Anti-CD81 (TAPA1) mAb (A103-10)
MEX003-4	Anti-CD81 (TAPA1) mAb-FITC (A103-10)
MEX003-6	Anti-CD81 (TAPA1) mAb-Biotin (A103-10)
MEX003-12	Anti-CD81 (TAPA1) mAb-ALP (A103-10)
MEX004-6	Anti-EpCAM mAb-Biotin (B8-4)
K0142-6	Anti-PSMA (Human) mAb-Biotin (107-1A4)
D281-6	Anti-CD61 (GPIIIa) (Human) mAb-Biotin (T74)
D252-3	Anti-CD9 (Human) mAb (10H6)
D252-5	Anti-CD9 (Human) mAb-PE (10H6)
D131-3	Anti-CD9 (Mouse) mAb (JF9)
D131-4	Anti-CD9 (Mouse) mAb-FITC (JF9)
D263-3	Anti-CD63 (LAMP-3) (Mouse) mAb (R5G2.1)
D082-3	Anti-CD151 (SFA-1) (Human) mAb (SFA1.2B4)
D082-5	Anti-CD151 (SFA-1) (Human) mAb-PE (SFA1.2B4)
D050-3	Anti-CD29 (Integrin β 1) (Human) mAb (AG89)
D050-5	Anti-CD29 (Integrin β 1) (Human) mAb-PE (AG89)
D276-3	Anti-CD36 (GPIV) (Human) mAb (GS95)
D276-A48	Anti-CD36 (GPIV) (Human) mAb -Alexa Fluor [®] 488 (GS95)
D276-A64	Anti-CD36 (GPIV) (Human) mAb -Alexa Fluor [®] 647 (GS95)
D269-3	Anti-EpCAM (CD326) (Mouse) mAb (2-17-F1)
M076-6	Mouse IgG2a (isotype control)-Biotin (6H3)
M076-12	Mouse IgG2a (isotype control)-ALP (6H3)

Kits

MEX-SA	ExoCap™ Streptavidin Kit
MEX-SA123	ExoCap™ Streptavidin CD9/CD63/CD81 Set
MEX-E	ExoCap™ Nucleic Acid Elution Buffer
MEX1001	ExoDiluent for Immunoassay
RN1001	RIP-Assay Kit

Others (reagents, equipments, etc.)

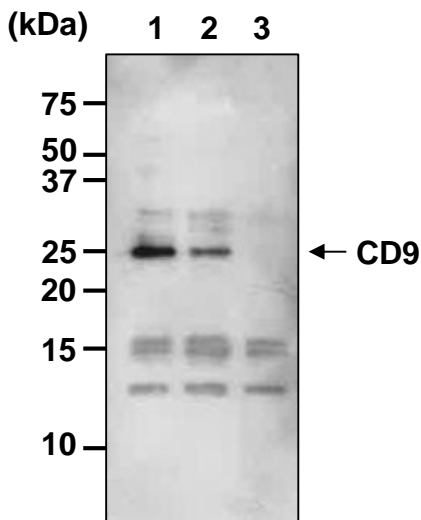
MJS002V2	Protein G-Magnetic Beads
3190	Magnetic Rack

Other related antibodies and kits are also available.
Please visit our website at <http://ruo.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 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% 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% MeOH). 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 blot analysis of CD9 protein

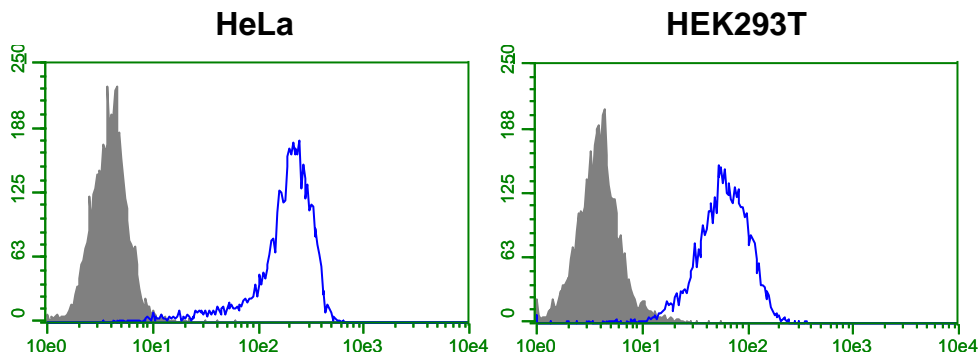
Lane 1: HeLa
Lane 2: HEK293T
Lane 3: WR19L

Immunoblotted with Anti-CD9 mAb-Biotin (MEX001-6)

Flow cytometric analysis

- 1) Wash the cells (5×10^5 cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Add 10 μ 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 μ L of the primary antibody at the concentration as suggested in the **APPLICATION** 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; HeLa and HEK293T)



Flow cytometric detection of human CD9

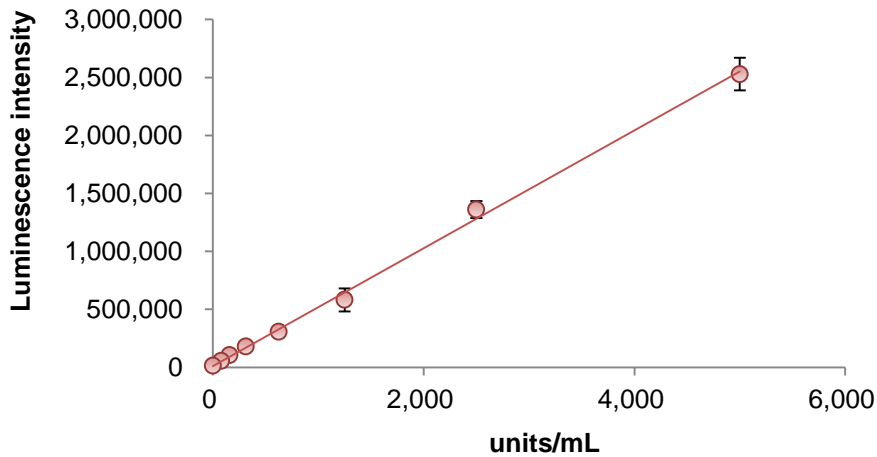
Left: HeLa

Right: HEK293T

Open: Anti-CD9 mAb-Biotin (MEX001-6)

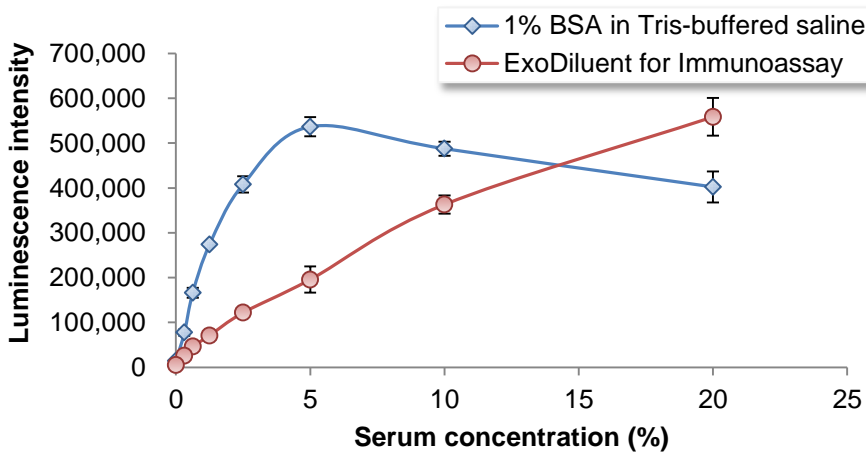
Closed: Mouse IgG2a (isotype control)-Biotin (M076-6)

Sandwich CLEIA (chemiluminescence enzyme immunoassay)



Sandwich CLEIA for measurement of CD9 expressed HeLa-derived exosomes

Sample: HeLa-derived exosomes prepared by ultracentrifugation
Capture Antibody: Anti-CD9 mAb (MEX001-3)
Detection Antibody: Anti-CD9 mAb-Biotin (MEX001-6)



Sandwich CLEIA for measurement of CD9 expressed exosomes from human serum

Sample: Pooled human serum from healthy volunteers
Capture Antibody: Anti-CD9 mAb (MEX001-3)
Detection Antibody: Anti-CD9 mAb-Biotin (MEX001-6)