

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 (<u>non-reducing condition</u>)
<u>Flow cytometry</u>	5 μ g/mL
<u>Sandwich CLEIA</u>	Can be used.
<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)

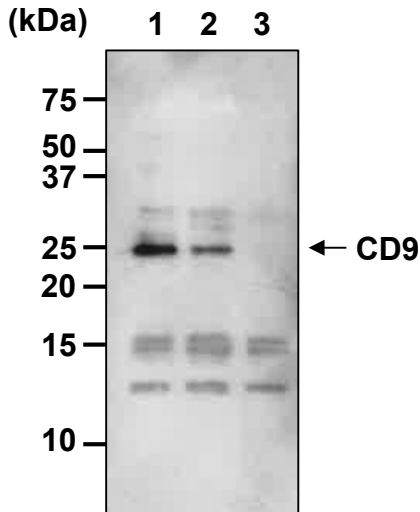
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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

- 1) Wash 1×10^7 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% 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 blot analysis of CD9 protein

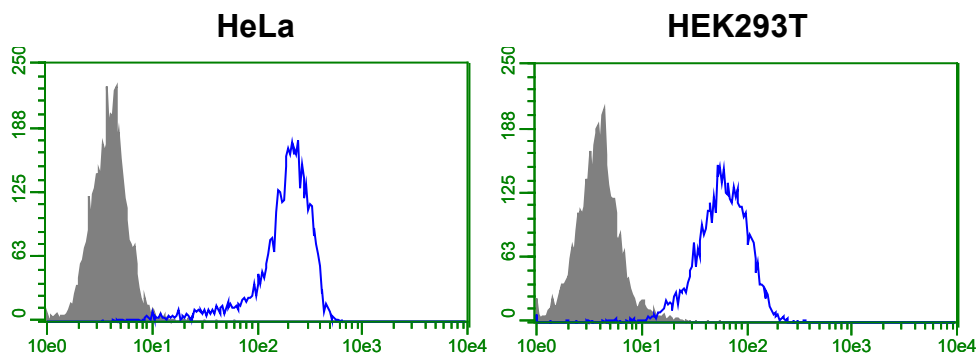
- Lane 1: HeLa
- Lane 2: HEK293T
- Lane 3: WR19L

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

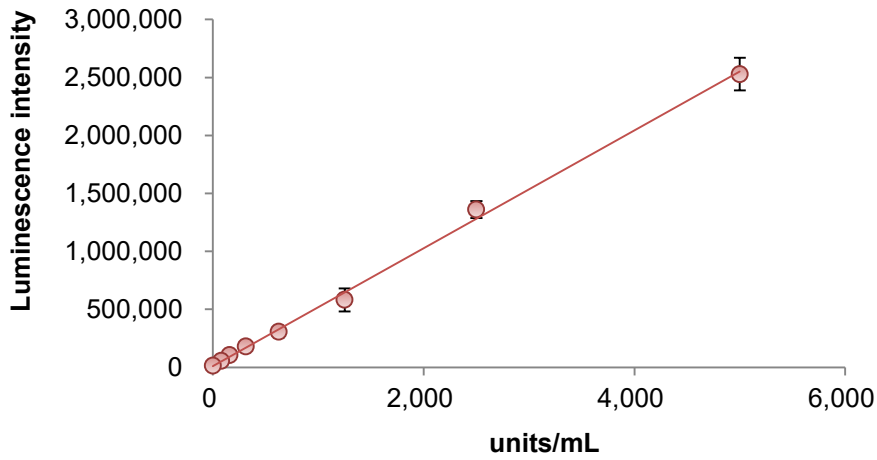
Left: HeLa

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)