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 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 (non-reducing condition)
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 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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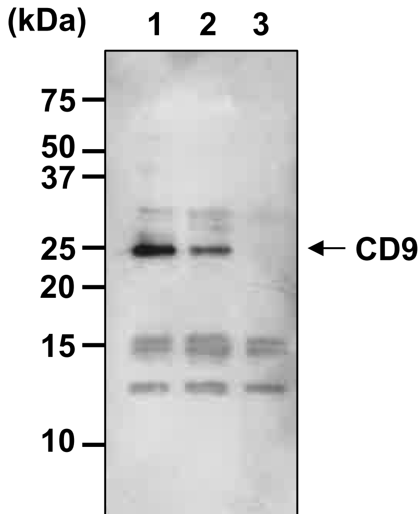
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 µL 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. × 3).
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).
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).
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

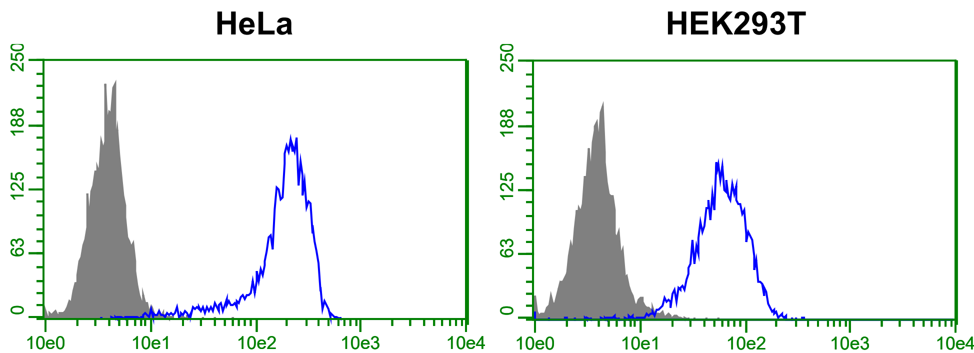
Lane 1: HeLa
Lane 2: HEK293T
Lane 3: WR19L

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

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) once 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 **APPLICATION** diluted with washing buffer.
4) Mix well and incubate for 20 min. at room temperature.
5) Wash the cells once 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 once 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)

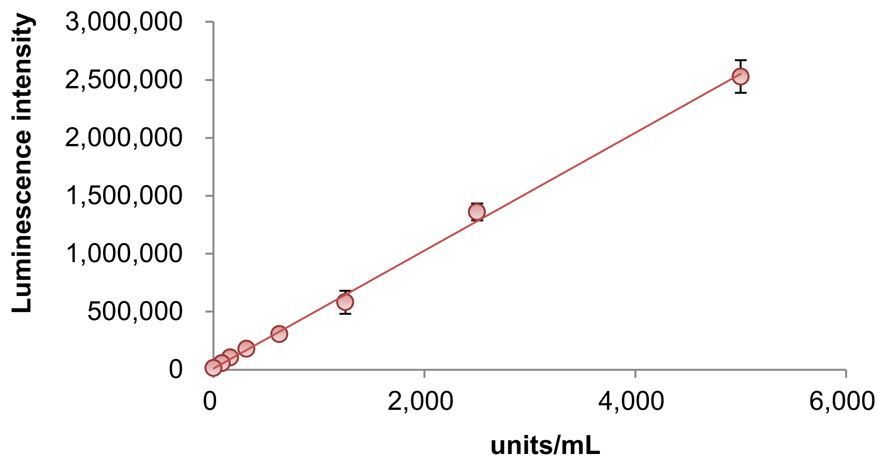


Flow cytometric detection of human CD9

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)