

MONOCLONAL ANTIBODY

Anti-CD9 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D252-3	10H6	Mouse IgG1 κ	100 μ L	1 mg/mL

BACKGROUND: CD9 is expressed on platelets, eosinophils, basophils, pre-B cells, activated T cells and neural cell lines. It belongs to a member of the tetraspanin transmembrane-protein (TM4) superfamily, which includes CD37, CD53, CD63, CD81, CD82, CD151 and CD231. Several members of this family, including CD9, form noncovalent associations with integrins, particularly β 1 integrins (CD29). Tetraspanins are involved in membrane events such as sperm-oocyte fusion, myoblast fusion, mononuclear phagocyte fusion, osteoclastogenesis, and paranodal junction formation in the peripheral nervous system. In addition, tetraspanins are implicated in viral processes such as CD63 in HIV infection, CD81 in hepatitis C virus infection, CD82 in cell-to-cell human T cell leukemia virus type I (HTLV-1) spreading, and CD9 in feline immunodeficiency virus (FIV) and canine distemper virus spreading.

SOURCE: This antibody was purified from hybridoma (clone 10H6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with human prostate carcinoma cell line (PC3).

FORMULATION: 100 μ g IgG in 100 μ L volume of 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 .

REACTIVITY: This antibody reacts with CD9 antigen on Western blotting and Flow cytometry.

APPLICATIONS:

Western blotting: 1 μ g/mL for chemiluminescence detection system (non-reducing condition)

Immunoprecipitation: Not tested

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Flow cytometry: 10 μ g/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

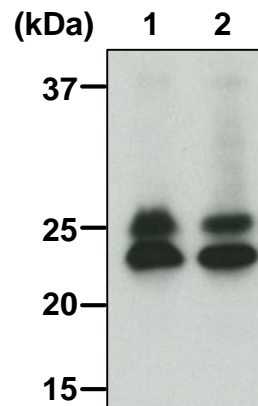
For Research Use Only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HL-60, lymphocyte, granulocyte, monocyte	Not tested	Not tested
Reactivity on FCM	+		

REFERENCE:

- 1) Boucheix, C., *et al.*, *J. Biol. Chem.* **266**, 117-122 (1991)



Western blot analysis of human CD9 expression in HeLa (1) and HeLa-derived exosomes (2) using D252-3 under non-reducing condition.

PROTOCOL:

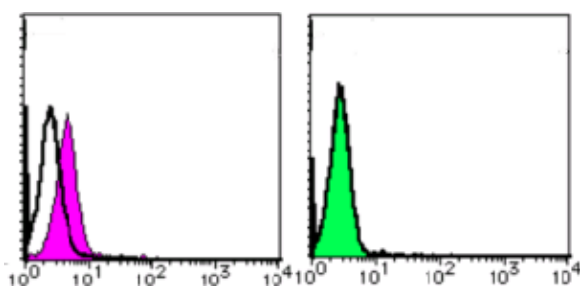
SDS-PAGE & Western Blotting

- 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 20 sec.)
- 2) Boil the samples for 5 minutes 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 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk in PBS overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted

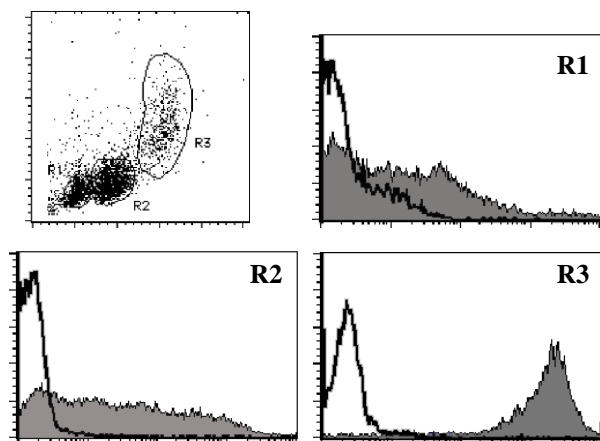
with 1% skimmed milk in PBS as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)

- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk in PBS for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 10) 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 1 minute.
- 12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa and HeLa-derived exosomes)



Flow cytometric analysis of CD9 expression on HL-60 cells (left) and HPB-ALL cells (right). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D252-3 to the cells.



Flow cytometric analysis of CD9 expression on human lymphocyte (R1), monocyte (R2) and granulocyte (R3) using D252-3. Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D252-3 to the cells.

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃].
- 2) Resuspend the cells with washing buffer (5 x 10⁶ cells/mL).
- 3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 4) Add 20 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 5 minutes at room temperature.
- 5) Add 20 µL of the primary antibody at the concentration as suggest in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 50 µL of 1:40 Anti-IgG (Mouse) pAb-PE (MBL; code no. IM-0551) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; HL-60)

RELATED PRODUCTS:

- 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)
- 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 β1) (Human) mAb (AG89)
- D050-5 Anti-CD29 (Integrin β1) (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)
- M075-3 Mouse IgG1 (isotype control) (2E12)