

MONOCLONAL ANTIBODY

FITC Labeled Mouse CD9

Code No.	Clone	Subclass	Quantity	Concentration
D131-4	JF9	Rat IgG2b	100 µL	500 µg/mL

BACKGROUND: CD9, a member of the tetraspanin or transmembrane 4 super family (TM4SF; CD37, CD53, CD63, CD81, CD82, etc) of proteins, is widely distributed on the surface of a variety of hematopoietic and epithelial cell types. Several members of the tetraspanin family, including CD9, form noncovalent associations with integrins, particularly $\beta 1$ integrins. CD9 and other tetraspanins have been postulated to participate in the regulation of cell growth, motility, and signaling via their physical association with integrins.

SOURCE: This antibody was purified from hybridoma (clone JF9) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Wister rat splenocyte immunized with the J774A.1 macrophage cells.

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA and 0.09% NaN₃.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with mouse CD9 on flow cytometry.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow Cytometry; 5 µg/mL (final concentration)

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

INTENDED USE:

For research use only. Not for clinical diagnosis.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	bone marrow cells	Not tested
Reactivity on FCM		+	

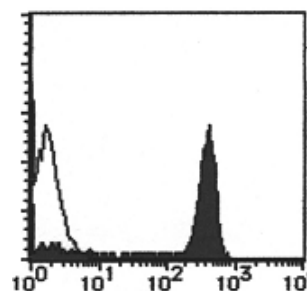
REFERENCE:

- 1) Chen, M.S., *et al.*, *Proc. Natl. Acad. Sci.* **96**, 11830-11835 (2002)

Clone JF9 is used in this reference.

RELATED PRODUCT:

D131-3 Mouse CD9 (JF9)



Flow cytometric analysis of mouse CD9 expression on mouse bone marrow cells. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D131-4 to the cells.

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step 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 (5x10⁶ 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 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN₃ to the cell pellet after tapping. Mix well and incubate for 10 minutes at room temperature.
- 5) Add 40 µL of the FITC labeled anti-mouse CD9 monoclonal antibody (JF9) (12.5 µg/mL) diluted with the washing buffer. Mix well and incubate for 20 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for flow cytometry: mouse bone marrow cells)