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

MONOCLONAL ANTIBODY

Mouse CD9

Code No.	Clone	Subclass	Quantity	Concentration
D131-3	JF9	Rat IgG2b	100 μg	1 mg/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 β1 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: 100 μg IgG in 100 μL 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 mouse CD9 on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 μg/mL (final concentration)

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

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

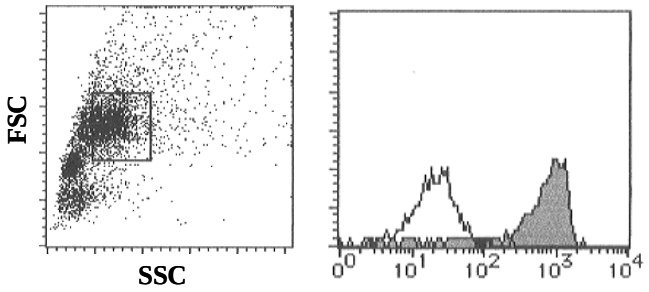
REFERENCE:

1) Chen, M.S., *et al*., *PNAS*. **96**, 11830-11835 (2002)

Clone JF9 is used in this reference.

RELATED PRODUCT:

D131-4 FITC labeled Mouse CD9 (JF9)



Flow cytometric analysis of CD9 expression on mouse born marrow cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D131-3 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% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ 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% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 μL of the anti-CD9 (JF9) (25 μg/mL) 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 30 μL of 1:40 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.

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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; mouse bone marrow cell)