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

MONOCLONAL ANTIBODY

Anti-mouse EpCAM/CD326

Code No.	Clone	Subclass	Quantity	Concentration
D269-3	2-17-F1	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: The mouse epithelial adhesion molecule (EpCAM) also known as gp40/CD326/Ly-74 is a 40-42 kDa of cell surface glycoprotein. EpCAM is a calcium-independent homotypic adhesion molecule. It is also expressed in thymic epithelial cells, thymic dendritic cells, peripheral T cells, intestinal epithelium, keratinocytes, lymph node, splenic dendritic cells and tumor-initiating cells. Recently, it is reported that EpCAM and TROP2, which is a member of the EpCAM family, is expressed in mouse hepatic oval cells. TROP2 is upregulated in oval cells raises the possibility that TROP2 modulate and/or enhance the intracellular signaling of EpCAM to promote proliferation and migration into liver parenchyma. The interaction of EpCAM and TROP2 may be involved in oval cell proliferation.

SOURCE: This antibody was purified from hybridoma (clone 2-17-F1) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with rat lymphocyte immunized with Ba/F3 cell transfectant overexpressing EpCAM cDNA.

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 EpCAM on Flow cytometry.

APPLICATIONS:

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

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	P19	Not Tested
Reactivity on WB		+	

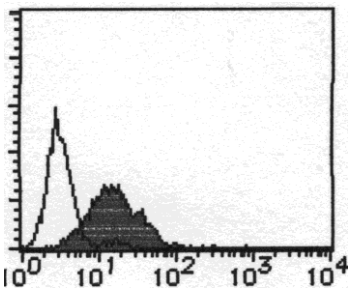
REFERENCES:

1) Okabe, M., *et al.*, *Development* **136**, 1951-1960 (2009)
2) Tanaka, M., *et al.*, *Mech. Dev.* **126**, 665-676 (2009)

Clone 2-17-F1 is used in these references.

RELATED PRODUCTS:

D187-3	anti-Dlk/Pref-1 (24-11)
D187-4	anti-Dlk/Pref-1-FITC (24-11)
D187-5	anti-Dlk/Pref-1-PE (24-11)
M081-3	Rat IgG2a isotype control (2H3)
M081-4	Rat IgG2a isotype control-FITC (2H3)
M081-5	Rat IgG2a isotype control-PE (2H3)



Flow cytometric analysis of mouse EpCAM expression on P19. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D269-3 to the cells.*

PROTOCOLS:

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% 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 20 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 20 μL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in 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 1:100 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) 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; P19)