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

MONOCLONAL ANTIBODY

Anti-Dlk (Pref-1) mAb-FITC

Code No.	Clone	Subclass	Quantity	Concentration
D187-4	24-11	Rat IgG1	100 µL	500 µg/mL

BACKGROUND: Delta like protein (Dlk), also known as Preadipocyte factor-1 (Pref-1) or zona glomerulosa-specific factor (ZOG), is an EGF-like transmembrane protein expressed preadipocytes but not in mature adipocytes. It is highly expressed in fetal liver, the adrenal gland, and placenta, as well as some neuroendocrine tumors and small cell lung carcinomas, where it plays a role in differentiation and proliferation. Dlk positively and negatively regulates adipocyte differentiation via at least four major variants (45-60 kDa) of Dlk generated by alternatively splicing. Constitutive expression of Dlk inhibits adipogenesis, but insulin or insulin like growth factor-1 (IGF-1) can circumvent this inhibition. Regulated processing of Dlk releases a 50 kDa soluble form that was previously characterized as Fetal Antigen-1, a protein involved in pancreatic island cell differentiation.

SOURCE: This antibody was purified from hybridoma (clone 24-11) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell with rat splenocyte immunized with Pref-1-Fc protein.

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA and 0.09% NaN_3.
*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 Dlk on Flow cytometry.

APPLICATION:
Flow cytometry; 50 µg/mL
*Please refer to the data sheet (MBL; code no. D187-3) for other applications.

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

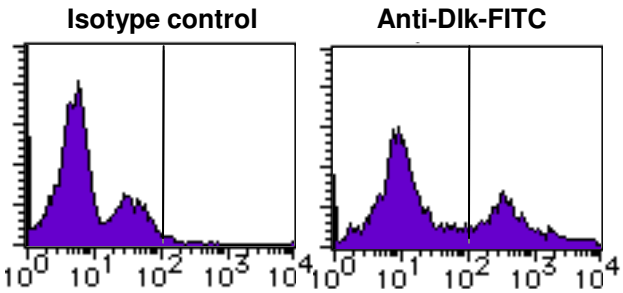
Species	Human	Mouse	Rat
Cell	Not tested	Fetal hepatocytes	Not tested
Reactivity on FCM		+	

REFERENCES:
1) Cheung, P. F., *et al., PLoS One* **6**, e28246 (2011) [FCM]
2) Khurana, S. and Mukhopadhyay, A., *Am. J. Pathol.* **173**, 1818-1827 (2008) [FCM]
3) Suzuki, K., *et al., Gastroenterology*, **135**, 270-281 (2008) [IHC]
4) Tanimizu, N., *et al., J. Cell Sci.* **116**, 1775-1786 (2003)
5) Kaneta, M., *et al., J. Immunol.* **164**, 256-264 (2000)

Clone 24-11 is used in the reference number 1)-3).

RELATED PRODUCTS:

D187-3	Anti-Dlk (Pref-1) mAb (24-11)
D187-5	Anti-Dlk (Pref-1) mAb-PE (24-11)
M080-3	Rat IgG1 (isotype control) (1H5)
M080-4	Rat IgG1 (isotype control)-FITC (1H5)
M080-5	Rat IgG1 (isotype control)-PE (1H5)



Flow cytometric analysis of Dlk expression on mouse fetal liver cells (E14) using isotype control (left) and D187-4.

This data was provided from Laboratory of Cell Growth and Differentiation, IMCB, The University of Tokyo.

PROTOCOL:
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].

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

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 primary antibody at the concentration of as suggested in the **APPLICATION** 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; mouse fetal hepatocytes, E14.5)