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

MBL
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MONOCLONAL ANTIBODY

Anti-Dlk (Pref-1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D187-3	24-11	Rat IgG1	100 µL	1 mg/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 Dlk-Fc fusion protein.

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 Dlk on Flow cytometry and Immunohistochemistry.

SPECIES CROSS REACTIVITY:

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

INTENDED USE:
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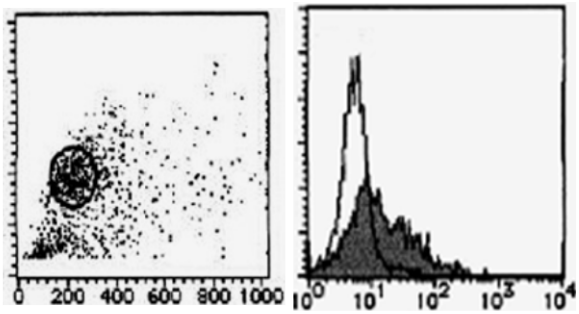
APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 2).
Immunohistochemistry (frozen section); 1 µg/mL
Immunohistochemistry (paraffin section); 10 µg/mL
Flow cytometry; 5-10 µg/mL

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

REFERENCES:
1) Tan, K. S., *et al., Biol. Open* **4**, 596-607 (2015) [IHC]
2) García-Alonso, V., *et al., J. Biol. Chem.* **288**, 28230-28242 (2013) [IC]
3) Cheung, P. F., *et al., PLoS One* **6**, e28246 (2011) [FCM]
4) Khurana, S. and M ukhopadhyay, A., *Am. J. Pathol.* **173**, 1818-1827 (2008) [FCM]
5) Suzuki, K., *et al., Gastroenterology* **135**, 270-281 (2008)
6) Tanimizu, N., *et al., J. Cell Sci.* **116**, 1775-1786 (2003)
7) Kaneta, M., *et al., J. Immunol.* **164**, 256-264 (2000)

Clone 24-11 is used in the reference number 1)-5).



Flow cytometric analysis of Dlk expression on Mouse Fetal Hepatocytes. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D187-3 to the cells.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

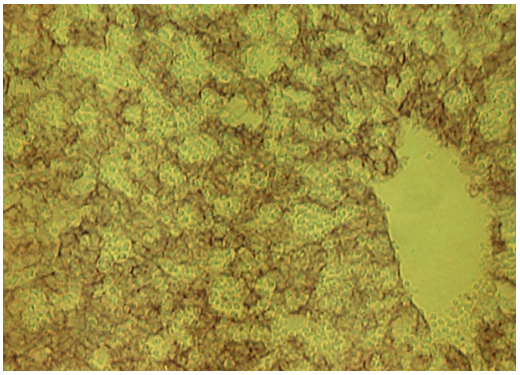
PROTOCOLS:

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 0.09% 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
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 0.09% 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 **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 PE conjugated anti-rat IgG antibody diluted with the washing buffer. Mix well and incubate for 15 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; Mouse fetal hepatocytes, E14.5)



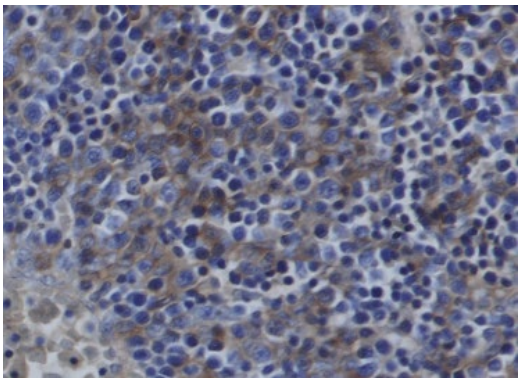
Immunohistochemical detection of Dlk on frozen section of mouse fetal liver (E14) with D187-3.

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

Immunohistochemical staining for frozen sections

1) Mouse fetal liver was embedded in OCT compound.
2) Make the frozen sections using Microtome.
3) Fix the sections with 4% paraformaldehyde in PBS.
4) Cover tissues with normal goat serum to block non-specific staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 3% normal goat serum as suggested in the **APPLICATIONS**.
6) Incubate the sections for 1 hour at room temperature.
7) Wash the slides 3 times in PBS for 5 minutes each.
8) Wipe gently around each section and cover tissues with Biotinylated secondary antibody (Vector). Incubate for 30 minutes at room temperature. Wash as in step 7).
9) Wipe gently around each section and cover tissues with Vectastain® ABC Kit (Vector). Incubate for 30 minutes at room temperature. Wash as in step 7).
10) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse fetal liver, E14)



Immunohistochemical detection of Dlk on paraffin embedded section of mouse fetal liver (E14.5) with D187-3.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes at room temperature (20~25°C) to block non-specific staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
6) Incubate the sections for 1 hour at room temperature.
7) Wash the slides 3 times in PBS for 5 minutes each.
8) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 1 hour at room temperature.

9) Wash the slides 3 times in PBS for 5 minutes each.
10) Visualize by reacting for 5 minutes with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes.
13) Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each. Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse fetal liver, E14.5)

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https://ruo.mbl.co.jp/.