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

MONOCLONAL ANTIBODY

Anti-mouse Lyve-1

Code No.	Clone	Subclass	Quantity	Concentration
D296-3	14-4	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: Lyve-1 (lymphatic vessel endothelial hyaluronan receptor 1) as known as HAR/Crsbp-1/ Xlkd1 is a receptor for HA (extracellular matrix glycosaminoglycan hyaluronan). The deduced amino acid sequence of mouse Lyve-1 predicts a 318-residue. Lyve-1 is a homologous to CD44 that binds both soluble and immobilized HA. Lyve-1 is predominantly expressed by lymphatic endothelial cells (LECs), and also by both fetal and adult Hepatic sinusoidal endothelial cells (HSECs). Lyve-1 is a powerful marker for lymphatic vessels.

SOURCE: This antibody was purified from hybridoma (clone 14-4) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with Wistar rat lymphocyte immunized with mouse fetal hepatic 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 Lyve-1 on Flow cytometry.

APPLICATIONS:

Immunohistochemistry; Not tested
It is reported that this monoclonal antibody can be used in Immunohistochemistry using frozen section in the reference number 1).

Flow cytometry; 10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	Transfectant	Not Tested
Reactivity on FCM		+	

INTENDED USE:
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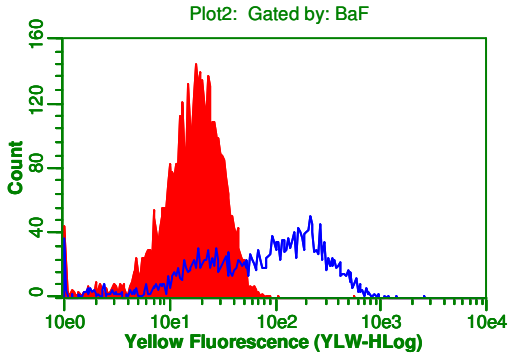
REFERENCES:

1) Nonaka, H., *et al.*, *Dev. Dyn.* **236**, 2258-2267 (2007)
2) Oliver, G., *Nat. Rev. Immunol.* **4**, 35-45 (2004)

Clone 14-4 is used in the reference number 1).



Flow cytometric analysis of mouse Lyve-1 expression on mouse Lyve-1 transfectant. *Closed histogram indicates the reaction of Isotypic control to the cells. Open histogram indicates the reaction of D296-3 to the cells.*

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)].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 100 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 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 40 µL of 1:50 PE conjugated anti-rat IgG (MBL; code no. IM-1623) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.

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e-mail support@mbl.co.jp, TEL 052-238-1904

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; transfectant)

RELATED PRODUCTS:

D225-3	Anti-mouse Lyve-1 (ALY7)
D225-5	Anti-mouse Lyve-1-PE (ALY7)
M081-3	Rat IgG2a isotype control (2H3)
IM-1623	Anti-rat IgG-PE (polyclonal)