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

MONOCLONAL ANTIBODY

Anti-LYVE-1 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D225-3	ALY7	Rat IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Hyaluronan (HA) is a high molecular weight polymer composed of alternating glucuronic acid and N-acetylglucosamine. HA is involved in homeostasis, development, and tissue remodeling. A major receptor for HA is designated as LYVE-1 (Lymphatic vessel endothelial hyaluronan receptor-1). LYVE-1 is a ~35 kDa integral membrane protein which is down-regulated in human liver cancer and cirrhosis. LYVE-1 localizes on the luminal face of the lymph wall, but is absent from blood vessels. Thus, anti-LYVE-1 antibody is a powerful tool for the identification of lymph vessels and for studies of lymphangiogenesis. LYVE-1 antibodies have been used successfully to distinguish lymphatic invasion by malignant tumor cells from blood vessel invasion.

SOURCE: This antibody was purified from hybridoma (clone ALY7) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell X63-Ag8 with Wister rat splenocyte immunized with the recombinant mouse LYVE-1 (1-228 aa) encoding the extracellular domain.

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 and Immunohistochemistry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry (for paraffin section); 10 µg/mL
  Heat treatment is necessary for paraffin embedded sections.
  Autoclave; 10 minutes at 110°C in 10 mM citrate buffer (pH 6.5)
Immunofluorescence; Not tested*
  *It is reported that this antibody can be used in immunofluorescence in the reference number 1)-5).
Immunocytochemistry; Not tested
Flow cytometry; 5 µg/mL (final concentration)

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

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

REFERENCES:

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7) Mishima, K., *et al., Mol. Biol. Cell* **18**, 1421-1429 (2007)
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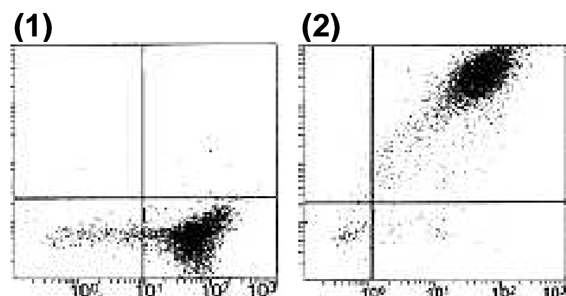

Flow cytometric analysis of Mouse LYVE-1 in Mouse LYVE-1-GFP transfected Ba/F3 cells. *The staining intensity of D225-3 (2) and Rat IgG1 (1) are shown in the vertical axis with GFP staining on the horizontal axis.*

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

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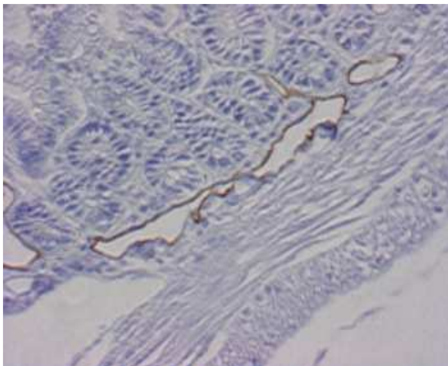
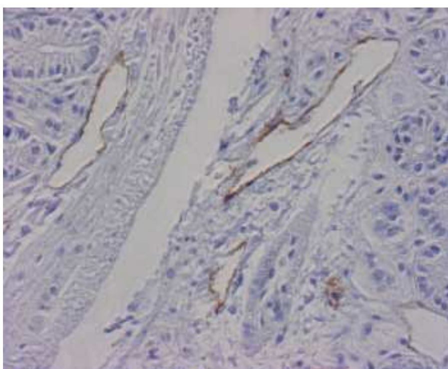
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

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; Transfectant)





Immunohistochemical detection of Mouse LYVE-1 on paraffin embedded section of lymphatic vessel in mouse intestine with D225-3.
These paraffin sections were kindly provided by Dr. Tohru Morisada, M.D. Ph.D. (The department of Cell Differentiation, The Sakaguchi Laboratory, Department of Obstetrics and Gynecology, School of Medicine, Keio University, Tokyo)

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) This section was made by AMeX (Acetone Methylbenzoate Xylene) method[5].
2) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
3) Wash the slides with Ethanol 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Autoclave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides with the autoclave for 10 minutes at 110°C. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Wash the slides with PBS 3 times for 3-5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
7) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
8) 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**.
9) Incubate the sections for 1 hour at room temperature.
10) Wash the slides 3 times in PBS for 5 minutes each.
11) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 10).
12) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 10).
13) 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.
14) Wash the slides in water for 5 minutes.
15) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
16) Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse lymphatic vessel)

RELATED PRODUCTS:

D225-5	Anti-LYVE-1 (Mouse) mAb-PE (ALY7)
D296-3	Anti-Lyve-1 (Mouse) mAb (14-4)
M080-3	Rat IgG1 (isotype control) (1H5)