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Inspiration for Life Science

MONOCLONAL ANTIBODY

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Catalog No.LKG-M009

Anti-Mouse LYVE-1 [Clone: 38M]

BACKGROUND

Cancer metastasis is associated with poor prognosis and accounts for the majority of cancer-related death[1,2]. There are two major mechanisms by which cancer metastasis occurs: hematogenous and lymphogenous metastasis[3]. The lymphatic route has been shown to be more important as an initial route for the spread of cancer than the hematogenous route[4,5], especially for carcinomas. Accordingly, metastatic spread to lymph nodes (LN) is regarded as a prognostic indicator[6].

Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) is a homolog of cluster of differentiation (CD) 44, a receptor for hyaluronan expressed on lymphatic endothelial cells (LEC)[7,8], and is utilized as a lymphatic-specific marker. LYVE-1 binds to hyaluronan, and is involved in the migration of LEC[9]. Furthermore, LYVE-1 promotes hyaluronan-induced lymphangiogenesis[9,10]. In clinical studies, LYVE-1 proteins were significantly increased in colon tumors compared with in unaffected colon tissues[11]. LYVE-1 gene expression was upregulated in muscle-invasive bladder cancers exhibiting positive lympho-vascular invasion and LN metastasis compared with in non-muscle invasive bladder cancers[12]. Thus, LYVE-1 is involved in primary tumor formation and metastasis, and it is expected to be useful for cancer treatment target.

This anti-LYVE-1 antibody (38M) specifically stain lymphatic vessels in several mouse tissues on immunohistology[13]. It has been developed by Cell Biology Laboratory, Kindai University (Prof. T. Masuko).

Product type	Primary Antibodies
Immunogen	Mouse LYVE-1 transfected cell
Raised in	Rat
Myeloma	P3 × 63Ag8.653
Clone number	38M
Isotype	IgG2a/κ
Source	Ascites
Purification	Caprylic acid clearance and ammonium sulphate precipitation
Buffer	0.9 % NaCl* *NOTE: This solution doesn't contain preservative. Preservative is added based on the research purpose.
Concentration	1 mg/mL
Volume	100 µL (100 µg)
Label	Unlabeled
Specificity	Mouse LYVE-1 extracellular domain 38M has no reactivity to mouse CD44 (the closest homologue of LYVE-1).
Cross reactivity	Mouse, No-cross reaction with rat or human, Other species are not tested.
Storage	Store cold (2 to 8 °C)

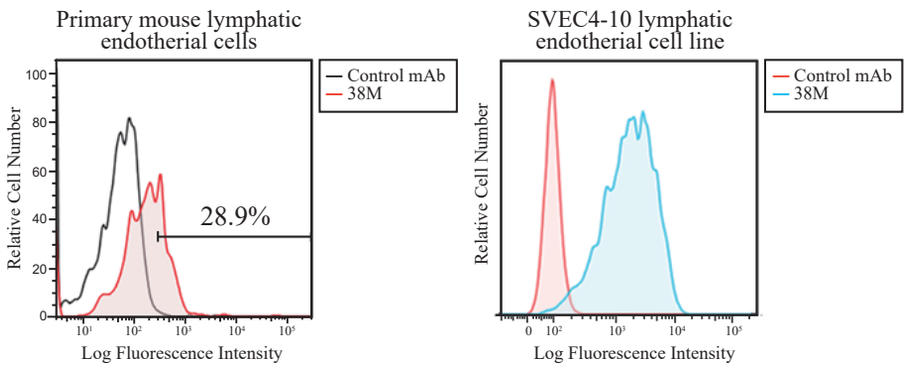
Application notes **Recommended dilutions**	Flow cytomerty; 10 µg/mL Immunohistochemistry (frozen); 10 µg/mL Immunoprecipitation - Other applications have not been tested. - Optimal dilutions/concentrations should be determined by the end user.

References

1) Mehlen P., et al., Nat Rev Cancer. 2006 Jun;6(6):449-58. PMID: 16723991
2) Nguyen DX., et al., Nat Rev Cancer. 2009 Apr;9(4):274-84. PMID: 19308067
3) Wong SY., et al., Cell Cycle. 2006 Apr;5(8):812-7. PMID: 16627996
4) Clarijs R., et al., J Pathol. 2001 Feb;193(2):143-6. PMID: 11180158
5) Pepper MS., et al., Clin Cancer Res. 2001 Mar;7(3):462-8. PMID: 11297234
6) Stacker SA., et al., FASEB J. 2002 Jul;16(9):922-34. PMID: 12087053
7) Banerji S., et al., J Cell Biol. 1999 Feb 22;144(4):789-801. PMID: 10037799
8) Prevo R., et al., J Biol Chem. 2001 Jun 1;276(22):19420-30. PMID: 11278811
9) Wu M., et al., PLoS One. 2014 Mar 25;9(3):e92857. PMID: 24667755
10) Yu M., et al., Exp Cell Res. 2015 Aug 1;336(1):150-7. PMID: 26116468
11) Langenes V., et al., Cancer Immunol Immunother. 2013 Nov;62(11):1687-95. PMID: 24013383
12) Poyet C., et al., Oncotarget. 2017 Mar 28;8(13):21871-21883. PMID: 28423532
13) Hara Y., et al., Cancer Sci. 2018 Oct;109(10):3171-3182. PMID: 30058195

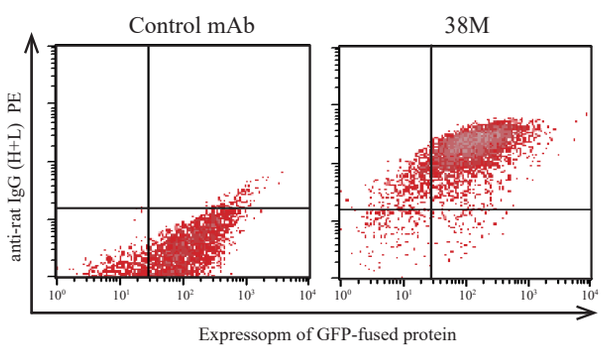
Application data

◆ Flow cytometry (FCM)



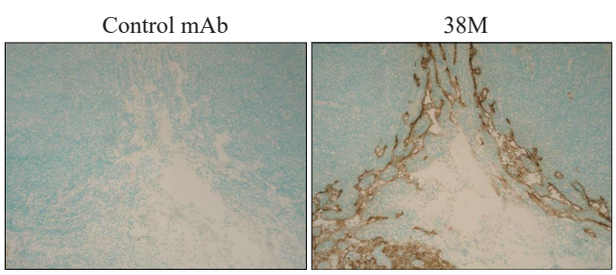
Flow cytometry analysis of mouse LYVE-1 in Primary mouse lymphatic endothelial cells (LEC) and SVEC4-10 LEC line with anti-LYVE-1 (38M, 10 µg/mL) antibody and PE-labeled anti Rat IgG antibody.

◆ Flow cytometry (FCM) **GFP-mouse LYVE-1** / HEK293



Flow cytometry analysis of antigenic specificity using mouse LYVE-1 expressing cells with anti-LYVE-1 (38M, 10 µg/mL) antibody and PE-labeled anti Rat IgG antibody. Specific response against GFP-fused mouse LYVE-1 protein by anti-LYVE-1 antibody was observed in a GFP intensity-dependent manner.

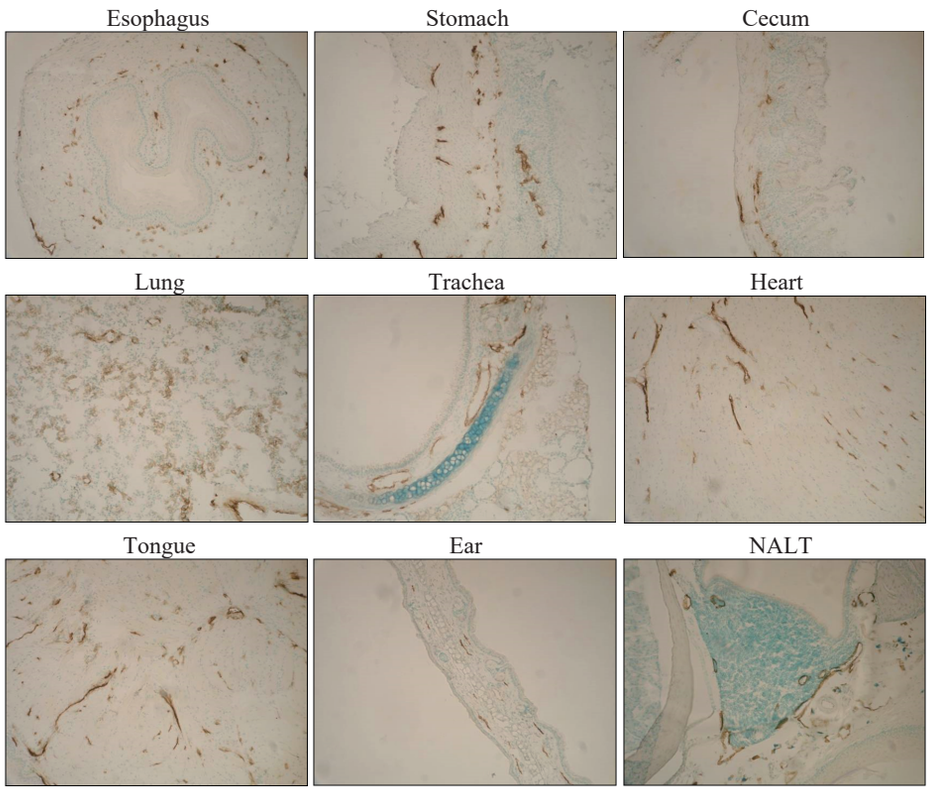
◆ Immunohistochemistry (Frozen) Mouse axillary lymph nodes



Immunohistochemistry staining mouse axillary lymph nodes with anti-LYVE-1 antibody (38M, 10 µg/mL). Nuclei were counterstained with methyl green.

Application data

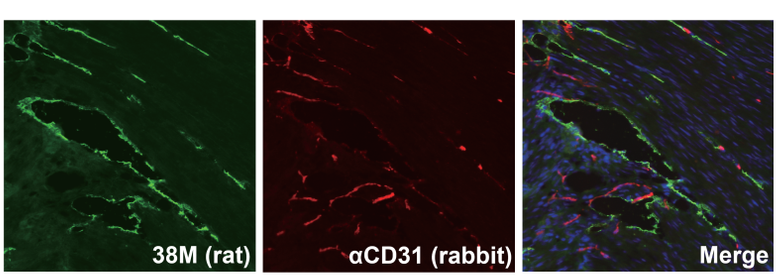
◆ Immunohistochemistry (Frozen)　Various mouse organs stained with 38M mAb



NALT, nasal-associated lymphoid tissue

Immunohistochemistry staining various mouse tissues with anti-LYVE-1 antibody (38M, 10 µg/mL), and then counterstained with methyl green. 38M reacted with lymphatic vessels in various mouse tissues.

◆ Immunohistochemistry (Frozen)　Confocal anlysis of LYVE-1 in mouse stomach tissue.



Mouse tissue sections were treated with anti-LYVE-1 antibody (38M) in combination with anti-CD31 rabbit antibody, and then treated with species-specific Alexa Fluor 488-conjugated anti-rat and Alexa Fluor 678-conjugated anti-rabbit IgG.

PROTOCOLS:

Flow cytometry (Cell Analyzing)

A. Cell Preparation

1. Remove cells from incubator.
2. Discard culture medium.
3. Briefly rinse the cell layer with PBS.
4. Add 0.25 % trypsin-EDTA solution to dish. Return the dish to the incubator and incubate for 2-10 minutes or until cells are detached.
5. Resuspend cells in complete growth medium with 10% FBS to inactivate the trypsin.

B. Staining

1. Aliquot $1x10^5$ cells into each assay tube.
2. Add 150 µl 0.2 % BSA in PBS to each tube and rinse by centrifugation.
3. Add 50 µl diluted primary antibody (10 µg/ml 38M in 0.2 % BSA in PBS) to the assay tubes.
4. Incubate for one hour at 4 °C.
5. Add 100 µl 0.1 % BSA in PBS to each tube and wash by centrifugation.
6. Wash two times in 150 µl 0.1 % BSA in PBS by centrifugation.
7. Resuspend cells in 50 µl PE-labeled secondary antibody solution (Jackson Immuno Research 712-116-153), diluted 1:200 in 0.1 % BSA in PBS.
8. Incubate for 30 minutes at 4 °C in the dark.
9. Add 100 µl 0.1 % BSA in PBS to each tube and wash by centrifugation.
10. Wash two times in 150 µl 0.1 % BSA in PBS by centrifugation
11. Resuspend cells in 100 µl PBS.
12. Analyze using flow cytometry.

Immunohistochemistry (Frozen section)

1. Isolate objective tissues from isoflurane anesthetized mice.
2. Embed the tissues in OCT Compound in liquid nitrogen.
3. Obtain tissue sections (7 µm) using a cryostat and mount them on poly-L-lysine (PLL)-coated slides.
4. Fixed Tissue sections by 4 % paraformaldehyde in PBS for 10 minutes at room temperature and treat with Block Ace overnight.
5. Incubate sections with diluted primary antibody (10 µg / mL 38M in 1 % BSA in PBS) for 60 minutes at room temperature.
6. Remove antibody solution and wash sections in PBS for 5 minutes.
7. Incubate sections in 3 % hydrogen peroxide in Methanol for 5 minutes to block endogenous peroxidase.
8. Wash sections in PBS for 5 minutes.
9. Incubate sections with 1:1000 diluted biotinylated rabbit anti-rat IgG (H+L; Jackson Immuno Research) in 1 % BSA in PBS for 60 minutes at room temperature.
10. Remove antibody solution and wash sections in PBS three times for 5 minutes each.
11. Wash sections in PBS three times for 5 minutes each.
12. Counterstain sections with Methyl Green (Merck, Darmstadt, Germany) per manufacturer's instructions.
13. Dehydrate sections:
    a. Incubate sections in 70 % ethanol for 30 seconds.
    b. Incubate sections in 90 % ethanol for 30 seconds.
    c. Incubate sections in 100 % ethanol for 30 seconds.
    d. Incubate sections in xylene three washes for 10 minutes each.
14. Mount coverslips.

RELATED PRODUCT

Product Name	Clone	Application	Quantity	Maker	Cat#
Anti Mouse LYVE-1	64R	FCM/IHC/IP/Functional assay	100 µg / 100 µL	CAC	LKG-M010

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