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MONOCLONAL ANTIBODY

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Catalog No.LKG-M010

Anti-Mouse LYVE-1 [Clone: 64R]

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	64R
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 64R 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)

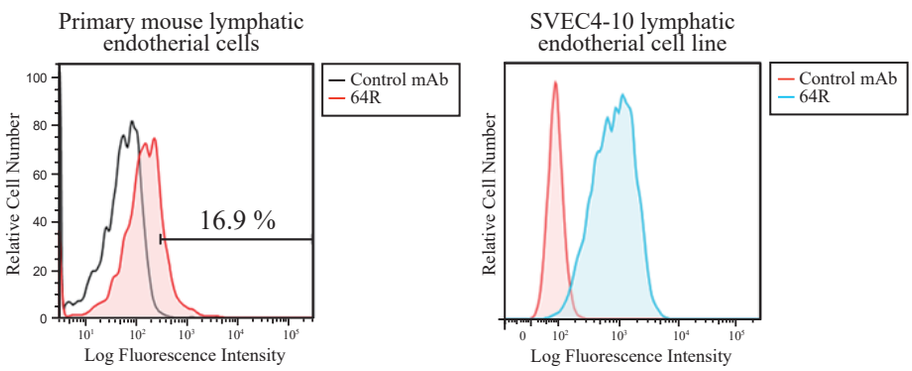
Application notes **Recommended dilutions**	Flow cytomerty; 10 µg/mL Tube formation assay; 10 µg/mL Wound healing assay; 10 µg/mL Immunohistochemistry (frozen); 10 µg/mL :38M (related product) is recommended for immunohistochemistry. Immunoprecipitation - Other applications have not been tested. - Optimal 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

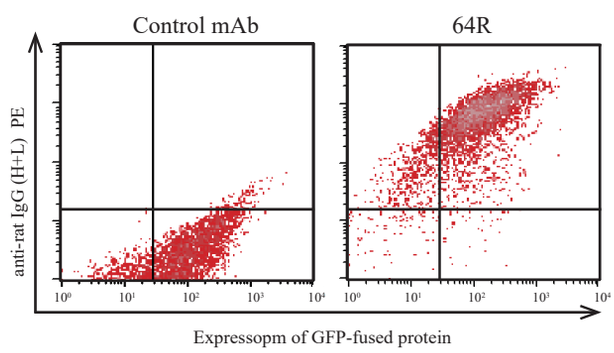
Application data

◆ Flow cytometry (FCM)



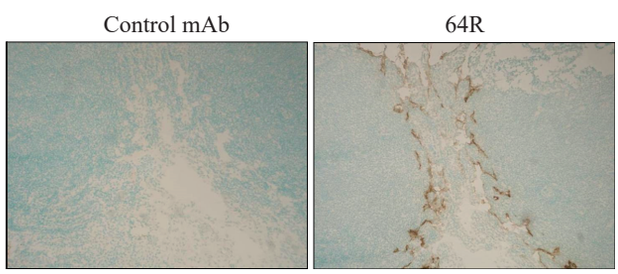
Flow cytometry analysis of mouse LYVE-1 in Primary mouse lymphatic endothelial cells (LEC) and SVEC4-10 LEC line with anti-LYVE-1 (64R, 10 µg/mL) antibody and PE-labeled anti Rat IgG antibody.

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



Flow cytometry analysis of antigenic specificity using mouse LYVE-1 expressing cells with anti-LYVE-1 (64R, 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.

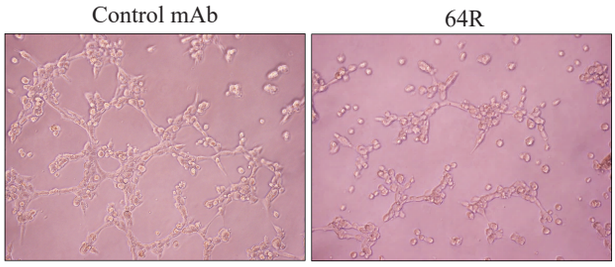
◆ Immunohistochemistry (Frozen) Mouse axillary lymph nodesh



Immunohistochemistry staining mouse axillary lymph nodes with anti-LYVE-1 antibody (64R, 10 µg/mL). Nuclei were counterstained with methyl green.
38M (related product) is recommended for immunohistochemistry, given that the immunostaining signal of 64R is weaker than those of 38M.

Application data

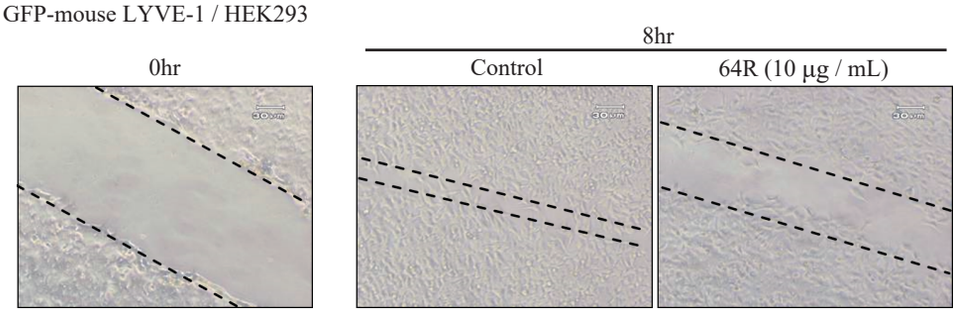
◆ Tube formation assay



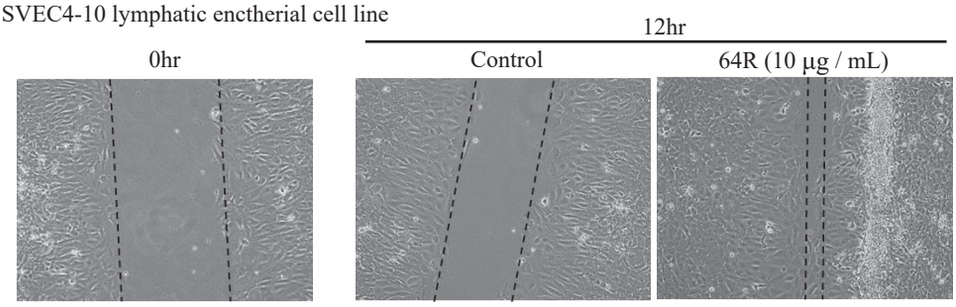
SVEC4-10 cells were plated onto Matrigel-coated chambers, added with or without anti-LYVE-1 antibody (64R, 10 μg/mL). Three hours after incubation at 37°C, images were taken using a microscope.

◆ Wound healing assay

A: GFP-mouse LYVE-1 / HEK293



B: SVEC4-10 lymphatic enctherial cell line



A: Representative images of wounds in HEK293 cells expressing mouse LYVE-1-GFP cell culture before and 8 hours after treatment with 64R. B: Representative images of wounds in SVEC4-10 cell culture before and 12 hours after treatment with 64R. Broken lines, wound edge.

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 64R 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.

PROTOCOLS:

Tube formation assay

A. Preparation of Matrigel-Coated slide chamber

1. Thaw Matrigel matrix (Corning, Corning, NY, USA) by submerging the vial in a 4°C refrigerator overnight before use.
2. Add Matrigel matrix to each well (100 µL) of an 8-well slide chamber (WATSON Bio Lab, Kobe, Japan), spread evenly with a pipet tip, and allow to solidify at 37°C for 30 minutes.

B. Cell Preparation

1. Remove lymphatic endothelial cells (LEC) from incubator.
2. Discard culture medium.
3. Briefly rinse the cell layer with PBS.
4. Trypsinize the cells to make a single-cell suspension, and then pellet the cells through centrifugation at 125 x g for 5 minutes at room temperature.
5. Re-suspend cells in complete growth medium with supernatants from MDA-MB-231-luc-LN cells.

C. Cell culture on Matrigel-Coated slide chamber

1. Plate cells onto Matrigel-coated chambers (5×10^4 cells), added with or without anti-LYVE-1 antibody (64R, 10 µg/mL).
   Note: The number of cells may need optimization depending on the growth properties of the cell line.
2. Incubate in humidified CO_2 incubators for 3 hours.
   Note: Incubation time may need optimization depending on the cell properties.
3. Observe cells under a microscope.

Wound healing assay

1. Plate cells onto tissue culture plate.
2. Make scratch wounds by scraping the cell layer with a micropipette tip, when the cells neared 100 % confluent.
3. After scratching, gently wash the well twice with medium to remove the detached cells.
4. Replenish the well with fresh medium with Supernatants from MDA-MB-231-luc-LN cells, added with or without anti-LYVE-1 antibody (64R, 10 µg/mL).
5. Incubate in humidified CO_2 incubators for 12 hours.
   Note: Incubation time may need optimization depending on the cell properties.
6. Observe scratch wound under a microscope.

RELATED PRODUCT

Product Name	Clone	Application	Quantity	Maker	Cat#
Anti Mouse LYVE-1	38M	FCM/IHC/IP	100 µg / 100 µL	CAC	LKG-M009

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