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DP3500 Polyclonal Antibody to LYVE-1 - Purified

Alternate names: CRSBP-1, CRSBP1, Cell surface retention sequence-binding protein 1, Extracellular link

domain-containing protein 1, HAR, Hyaluronic acid receptor, LYVE1, Lymphatic vessel

endothelial hyaluronic acid receptor 1, XLKD1

Quantity: 0.1 mg

Background: LYVE-1 has been identified as a major receptor for HA (extracellular matrix

glycosamino-glycan hyaluronan) on the lymph vessel wall. The deduced amino acid sequence of LYVE-1 predicts a 322-residue type I integral membrane polypeptide 41% similar to the CD44 HA receptor with a 212-residue extracellular domain containing a single Link module the prototypic HA binding domain of the Link protein superfamily. Like CD44, the LYVE-1 molecule binds both soluble and immobilized HA. However, unlike CD44, the LYVE-1 molecule colocalizes with HA on the luminal face of the lymph vessel wall and is completely absent from blood vessels. Hence, LYVE-1 is the first lymph-specific HA receptor to be characterized and is a uniquely powerful marker for

lymph vessels themselves.

Uniprot ID: <u>Q9Y5Y7</u>

NCBI: NP 006682.2

GeneID: 10894
Host: Rabbit

Immunogen: Recombinant Human soluble LYVE-1 (24-232) produced in insect cells.

Remarks: The recombinant soluble LYVE-1 consists of amino acid 24 (Ser) to 232 (Gly)

and is fused to a C-terminal His-tag (6xHis).

Format: State: Lyophilized purified IgG fraction

Purification: Protein A Chromatography (+ his tag depleted)

Buffer System: 5mM PBS, pH 7.2 without preservatives or stabilizers

Reconstitution: Restore in sterile water to a concentration of 0.1-1.0 mg/ml.

Applications: ELISA (1-15 μ g/ml).

Western blot (1-2 μ g/ml). FACS analysis (3-20 μ g/ml).

Immunohistochemistry on Frozen Sections (6-30 μg/ml).

Please Note: For use on Paraffin Embedded Sections the Affinity Purified antibody

(Cat.-No DP3500PS) is recommended.

Other applications not tested. Optimal dilutions are dependent on conditions and

should be determined by the user.

Specificity: The antibody recognizes LYVE-1.

Species Reactivity: Tested: Human.

DP3500: Polyclonal Antibody to LYVE-1 - Purified

Storage:

The lyophilized IgG is stable at 2-8°C for one month or at -20°C for one year from

despatch.

When reconstituted the antibody is stable for six weeks at 2-8°C and at -20°C for

longer.

Aliquot to avoid repeated freezing and thawing.

Product Citations:

Purchased from Acris:

1. Geissler HJ, Dashkevich A, Fischer UM, Fries JW, Kuhn-Régnier F, Addicks K, et al. First year changes of myocardial lymphatic endothelial markers in heart transplant recipients. Eur J Cardiothorac Surg. 2006 May;29(5):767-71. Epub 2006 Jan 24. PubMed PMID: 16439147.

2. Kimura H, Miyashita H, Suzuki Y, Kobayashi M, Watanabe K, Sonoda H, et al. Distinctive localization and opposed roles of vasohibin-1 and vasohibin-2 in the regulation of angiogenesis. Blood. 2009 May 7;113(19):4810-8. doi:

10.1182/blood-2008-07-170316. Epub 2009 Feb 9. PubMed PMID: 19204325.

General Readings:

1. Mouta Carreira C, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Tomarev SI, et al. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. Cancer Res. 2001 Nov 15;61(22):8079-84. PubMed PMID: 11719431.

2. Jackson DG. The lymphatics revisited: new perspectives from the hyaluronan receptor LYVE-1. Trends Cardiovasc Med. 2003 Jan;13(1):1-7. PubMed PMID: 12554094.

3. Sleeman JP, Krishnan J, Kirkin V, Baumann P. Markers for the lymphatic endothelium: in search of the holy grail? Microsc Res Tech. 2001 Oct 15;55(2):61-9.

PubMed PMID: 11596151.

4. Mäkinen T, Veikkola T, Mustjoki S, Karpanen T, Catimel B, Nice EC, et al. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. EMBO J. 2001 Sep 3;20(17):4762-73. PubMed PMID: 11532940.

Protocols:

Double staining LYVE-1/CD31 on Humanen Cryo-Sections

- dry Cryo sections for 1h at RT
- fix sections for 10 min at -20°C in MeOH
- wash 3x3 min in PBS at RT
- block 20 min in 10% goat serum in PBS
- 1. AB over night at 4°C in 10% goat serum in PBS [anti h -LYVE-1 10µg/ml and anti h-CD31 1:50 (Klon JC70A)]
- wash 3x3 min in PBS at RT
- 2. AB 30 min at RT in PBS

[goat anti rabbit IgG (H+L) CY3 1:500 and goat anti mouse biotin 1:500]

- wash 3x3 min in PBS at RT
- staining of nucleus 10 min at RT in Hoechst Dye 1:5000 in PBS
- wash 3x3 min in PBS at RT
- cover with DAKO Fluorescent Mountingmedium

Staining protocol for Rabbit polyclonal anti-LYVE-1 on Paraffin Sections (Human Spleen)

The specimens (maximum edge length 0.5cm) are fixed for 24 hours (or longer) in



3.7% formalin in tap water (low pH during fixation is advantageous). Embedding is done in paraffin carefully avoiding temperatures exceeding 60°C. The sections are cut and deposited on silanized slides and dried at 60°C overnight. They are then deparaffinized and pretreated with protease XIV (Sigma No. P-5147) at 0.5mg/ml in TBS pH7.6 for 15min at room temperature. The primary polyclonal antibodies are applied at 1:100 to 1:150 in

PBS/1%BSA/0.1%NaN3 overnight.

Subsequently, the Vector anti-rabbit ABC kit for peroxidase is used according to the manufacturer's instructions and the presence of peroxidase revealed by diaminobenzidine reaction.

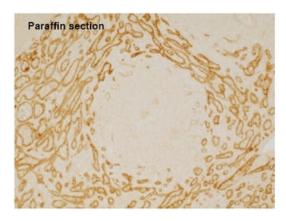
Note: The antibodies have to be used at a tenfold higher concentration on paraffin sections in comparison to cryo sections. High temperature antigen retrieval does not work, but protease pretreatment is mandatory.

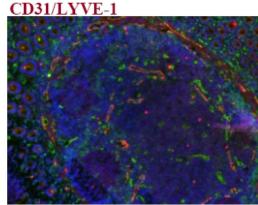
The protocol was established at the laboratory of:

Prof. Dr. Birte Steiniger Institute of Anatomy and Cell Biology Robert-Koch-Str.8 D-35037 Marburg Germany

E-mail: steinigb@staff.uni-marburg.de

DP3500 LYVE-1 antibody staining of Human Spleen Paraffin section. The experiment was performed by Prof. Dr. Birte Steiniger, Institute of Anatomy and Cell Biology Robert-Koch-Str. 8, D-35037 Marburg, Germany.



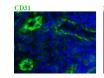


Pictures:



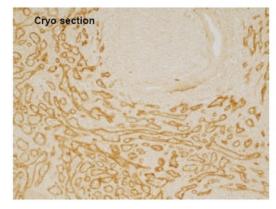
The staining was performed on cryosections from human colon carcinoma using polyclonal anti-Human LYVE-1 antibody DP3500/DP3500X (Protein-A purified, 10µg/ml). The experiments were perfomed by Dr. Ulrike Fiedler and Stefanie Koidel, Dept. of Vascular Biology and Angiogenesis Research Tumor Biology Center, Breisacher Str. 117, D-79106 Freiburg, Germany

DP3500 LYVE-1 antibody staining of Human Spleen Cryo Section. The experiment was performed by Prof. Dr. Birte Steiniger, Institute of Anatomy and Cell Biology Robert-Koch-Str. 8, D-35037 Marburg, Germany.

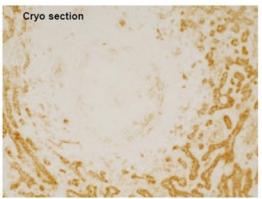






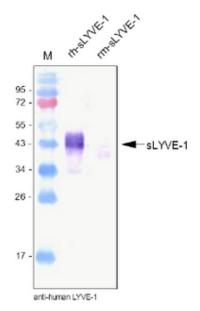


DP3500 LYVE-1 antibody staining of Human Spleen Cryo Section. The experiment was performed by Prof. Dr. Birte Steiniger, Institute of Anatomy and Cell Biology Robert-Koch-Str. 8, D-35037 Marburg, Germany.





Western analysis of recombinant Human sLYVE-1 and Mouse sLYVE-1 using an anti-Human LYVE-1 Antibody Cat.-No DP3500X directed against the extracellular domain of human LYVE-1. There is more or less no cross reactivity with Mouse LYVE-1.



FACS analysis with LYVE-1 antibody in HDMVECs

