

## Polyclonal Antibody to LYVE-1 - Purified

<b>Alternate names:</b>	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
<b>Catalog No.:</b>	DP3500X
<b>Quantity:</b>	0.2 mg
<b>Background:</b>	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.
<b>Uniprot ID:</b>	<a href="#">Q9Y5Y7</a>
<b>NCBI:</b>	<a href="#">NP_006682.2</a>
<b>GeneID:</b>	<a href="#">10894</a>
<b>Host:</b>	Rabbit
<b>Immunogen:</b>	Recombinant Human soluble LYVE-1 (24-232) produced in insect cells. <b>Remarks:</b> The recombinant soluble LYVE-1 consists of amino acid 24 (Ser) to 232 (Gly) and is fused to a C-terminal His-tag (6xHis).
<b>Format:</b>	<b>State:</b> Lyophilized purified IgG fraction <b>Purification:</b> Protein A Chromatography (+ his tag depleted) <b>Buffer System:</b> PBS, pH 7.4 without preservatives or stabilizers <b>Reconstitution:</b> Restore in sterile water to a concentration of > 0.5 mg/ml.
<b>Applications:</b>	<b>ELISA</b> (1-15 µg/ml). <b>Western blot</b> (1-2 µg/ml). <b>FACS analysis</b> (3-20 µg/ml). <b>Immunohistochemistry on Frozen Sections</b> (6-30 µg/ml). <b>Please Note:</b> For use on Paraffin Embedded Sections the affinity-purified antibody (DP3500PS) is recommended. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
<b>Specificity:</b>	The antibody recognizes LYVE-1.
<b>Species Reactivity:</b>	<b>Tested:</b> Human.

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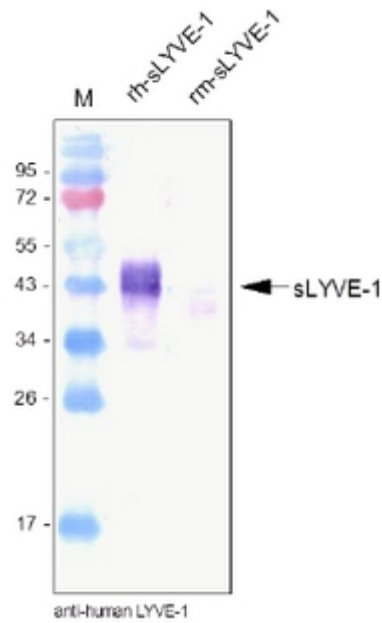
- Storage:** The lyophilized IgG is stable at 2-8°C for one month or at -20°C for one year from despatch. When reconstituted the antibody is stable for six weeks at 2-8°C and at -20°C for longer. Aliquot to avoid repeated freezing and thawing.
- Product Citation:**
1. Hans J. Geissler, Alexey Dashkevich, Uwe M. Fischer, Jochen W.U. Fries, Ferdinand Kuhn-Régnier, Klaus Addicks, Uwe Mehlhorn, and Wilhelm Bloch. First year changes of myocardial lymphatic endothelial markers in heart transplant recipients. Eur. J. Cardiothorac. Surg., May 2006; 29: 767-771.
  2. Hiroshi Kimura, Hiroki Miyashita, Yasuhiro Suzuki, Miho Kobayashi, Kazuhide Watanabe, Hikaru Sonoda, Hideki Ohta, Takashi Fujiwara, Tooru Shimosegawa, and Yasufumi Sato. Distinctive localization and opposed roles of vasohibin-1 and vasohibin-2 in the regulation of angiogenesis. Blood, May 2009; 113: 4810-4818.
- General References:**
1. Carriera et al., Cancer Res 61:8079, 2001.
  2. Jackson DG Trends Cardiovasc Med 13:1, 2003.
  3. Sleeman et al., Microsc Res Tech 55:61, 2001.
  4. Mäkinen et al., EMBO J 20: 4762, 2001.
- 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 de-paraffinized 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%NaN<sub>3</sub> 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
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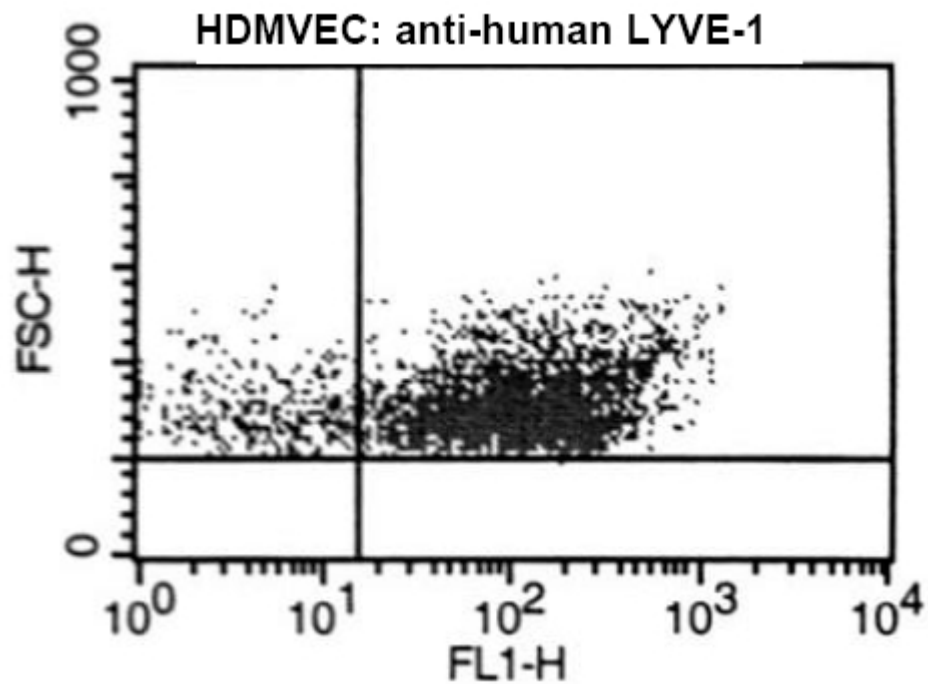
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Pictures:



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.

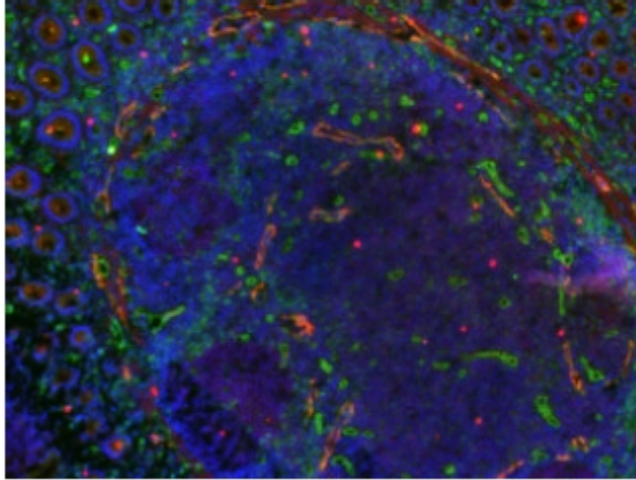
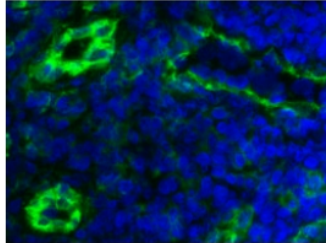
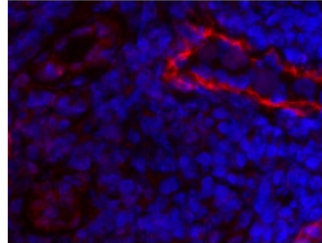
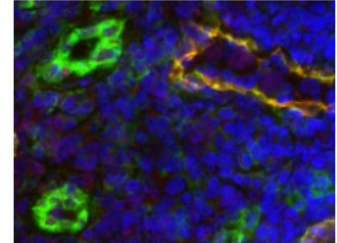


FACS analysis with LYVE-1 antibody in HDMVECs

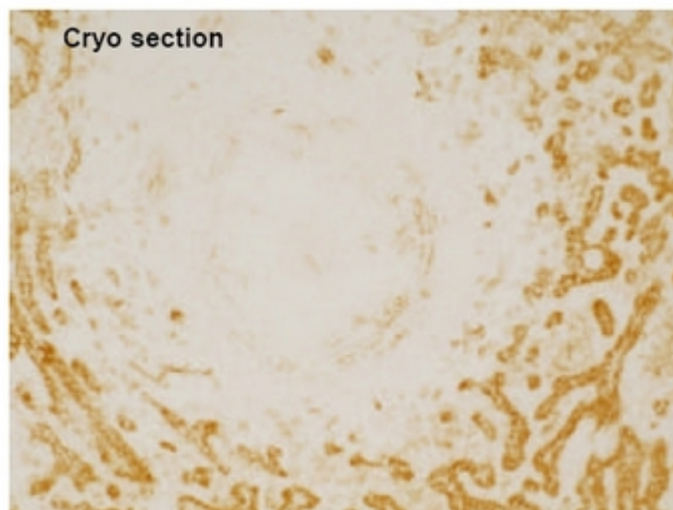
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**CD31/LYVE-1****CD31****LYVE-1****CD31/LYVE-1**

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 performed by Dr. Ulrike Fiedler and Stefanie Koidel, Dept. of Vascular Biology and Angiogenesis Research Tumor Biology Center, Breisacher Str. 117, D-79106 Freiburg, Germany

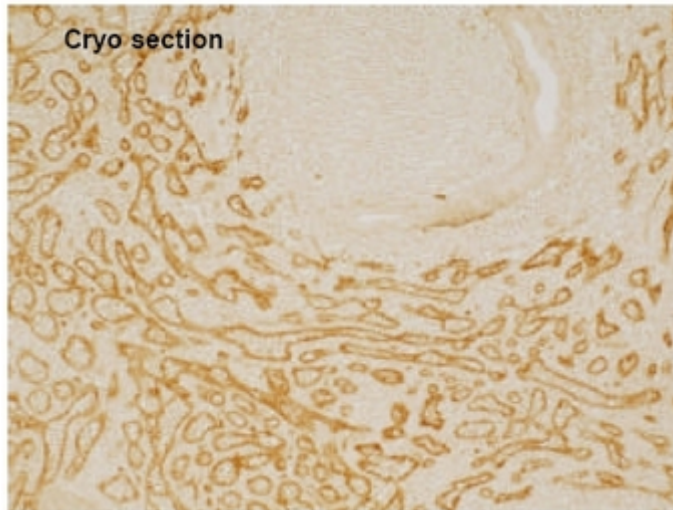


DP3500 LYVE-1 antibody staining of Human Spleen Cryo Section.  
The experiment was performed by Prof. Dr. Birte Steiniger, Institute of Anatomy and Cell Biology Robert-Koch-Str. 8, D-35037 Marburg, Germany.

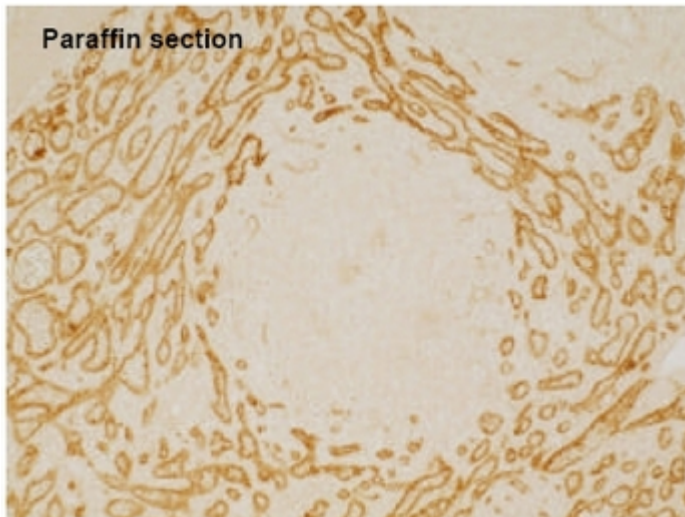
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DP3500 LYVE-1 antibody staining of Human Spleen Cryo Section.  
The experiment was performed by Prof. Dr. Birte Steiniger, Institute of Anatomy and Cell Biology Robert-Koch-Str. 8, D-35037 Marburg, Germany.



DP3500 LYVE-1 antibody staining of Human Spleen 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.

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