

**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:

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