Protocol for LYVE-1 staining on Prostate Sections

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Below is a protocol we use to stain frozen sections of the prostate. This procedure has been adapted from multiple protocols and altered to best fit our studies. It has worked fine in other tissues such as brain, lymph node, and B16 melanoma. Controls for blood vessel and immune cell staining using isotype conjugated antibodies are negative. For LYVE-1 controls, we chose to use secondary antibody alone due to lack of a non-specific rabbit polyclonal antibody.

1. We used 4 µm cryostat generated frozen sections of the prostate, a tissue we know contains LYVE-1 positive lymphatics and cells (submitted manuscript). The samples were unfixed, as we have determined that the fixing process degrades certain antigens of interest. The sections are placed on poly-L-lysine treated slides to help with the adherence of the tissue section to the slide.

2. The samples were blocked by adding 100 µl of phosphate buffered saline (PBS) + 1% bovine serum albumin + .1% sodium azide + 5% normal mouse serum (NMS)----all abbreviated PBA/NMS-----along with 5 µg/ml of Fc Block (Clone 2.4G2, BD PharMingen). The samples were incubated in a humidified box at 4 degrees C for 10 minutes. The samples were then washed by aspirating off the blocking mixture and adding 100 µl of PBA to each section. This wash step was repeated 3 times.

3. The primary antibodies used were: blood vessels; CD31-FITC (Clone MEC 13.3, BD PharMingen), immune cells; CD45-APC (Clone 30-F11, BD PharMingen), and lymphatic/cells; LYVE-1 (**RELIATech, #103-PA50**). These were added to the tissue samples each at a concentration of 3 µg/ml in 100 µl PBA/NMS, and the sections incubated in a humidified box at 4 degrees C for 25 minutes.

4. The sections were washed by aspirating off the primary antibody mixture, adding 100 µl of PBA and incubating for 3-5 minutes. This wash step was repeated 4-5 times.

5. The LYVE-1 antibody was detected by adding secondary antibody (mouse-anti-rabbit-Cy3, from Jackson ImmunoResearch Laboratories, Inc.) at 5 µg/ml in 100 µl PBA/NMS, and incubating as in step 3.

6. The sections were washed as described in Step 4.

7. Coverslips were added and the sections visualized using 3 color fluorescent microscopy.