Protocol for LYVE-1 staining on Ear using Whole Mount Histology

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Below is a protocol we developed in our laboratory, which is used to stain large pieces of tissue and visualize complete structures. You'll notice that the vasculature looks very different in this type of preparation when compared to frozen sections, as the whole mount preparation allows one to see the complete blood or lymphatic vessels. Below is the exact protocol we used to stain the ear samples, whose images are provided.

1. A 5mm x 5mm piece of the outer ear flap was placed into a 5ml polypropylene tube along with 200 µl of PBS + 1% bovine serum albumin + .1% sodium azide + 5% normal mouse serum (NMS)---(PBA/NMS).

2. The tissue was blocked using Fc-block (Pharmingen #553142) at 8ug/ml added directly to the 200ul of PBA/NMS containing the tissue, and incubated at 4 degrees C for 10 minutes.

3. To stain for blood vessels within the ear we used anti-CD31 (clone MEC 13.3, Pharmingen #553373), and to stain for lymphatic vessels we used anti-murine LYVE-1 (**RELIATech; #103-PA50**). The anti-CD31-FITC and anti-LYVE-1 antibodies were added directly to the mixture of 200 µl PBA/NMS/Fc-block at 4ug/ml and 8ug/ml respectively. (This concentration of LYVE-1 is quite high and probably could be titrated down considerably!). Samples were incubated on a shaker at 4 degrees C for a minimum of 1 hour.

4. Samples were washed by filling up the tube with 4mls of PBA and rotated at 4 degrees for 10-20 minutes.

5. After the first wash step, the PBA was drained and mouse anti rabbit-Cy3 (Jackson ImmunoResearch Labs) was used to detect the bound LYVE-1 antibody by adding the secondary antibody at 5 µg/ml to 200 µl of PBA/NMS and incubating as in step 3.

6. The samples were washed as described in step 4.

7. The tissue was mounted by placing it on a glass slide using forceps and 100ul of PBA was added to the tissue. A cover slip was then placed over the tissue and gently pressed down. Samples were visualized using 2-color fluorescent microscopy.