

### **Mouse PECAM-1 (CD31) fluorescent detection**

1. Snap freeze tissue embedded in O.C.T. compound. Store at -80.
2. Cut frozen 8-10 um sections and mount onto + charged slides, air dry 10-15 minutes, and store at -20 or -80 until ready to begin antibody stain.
3. Air dry slides 30 minutes at room temp, lay flat on bench.
4. Fix sections in ice cold acetone (dunk in slide holder), 5 minutes, air dry 30 minutes.
5. Wash slides in PBS (dunk), 2 times, 5 min each
6. Lay slides out face up in humid chamber. Block 30 minutes in Goat Block (2% normal goat serum in PBTT (0.1% TX-100, 0.05% T-20) + 1% BSA)
7. Aspirate off block and add rat anti-murine CD31/PECAM-1 (cat # 553370, Pharmingen) at 1:100 in PBS + 1% BSA, and incubate 30 minutes at RT.
8. Wash 2X in PBS (dunk), 5 min ea.
9. Secondary Ab: goat anti-rat IgG-FITC (sc-2011, 200 ug/0.5 ml, Santa Cruz Biotechnology) at 1:200 in PBS + 1% BSA.
10. Leave slides face up, aspirate off 2dary, and wash in PBS, 2X, 5 min.
11. Incubate in DAPI (5 ug/ml in PBS) 5 min.
12. Wash (dunk) in PBS, 3X, 5 min ea.
13. Mount in Fluoroguard and store slides at -20 until viewing.