

PECAM Protocol (cell chamber slides)

A. Zovein.. adapted from S. Jilani

- 1) 4% PFA : 1-2hr fix
- 2) Blocking with 5% Donkey Serum + 0.3% Triton, RT 2+hrs
- 3) 1:500 rat anti-mouse CD31+ (PECAM) in 1X Blocking Buffer
- 4) Incubate in humidified chamber overnight 4°C
- 5) PBS wash 5min x 3
- 6) 1:200 Donkey conjugated anti-rat Cyn3 in 1X Blocking buffer
- 7) Incubate RT x 2hrs
- 8) PBS wash 10min x 3
- 9) Topro3 (blue) 1ul of Topro3 in 500ul Vectashield

Brightfield vibratome modifications\* (per J. Alva)

Secondary: donkey anti-rat HRP 1:200 for 2hrs at RT in 1X blockbuffer

Rinse PBS 2-4hrs RT

Use DAB/NovaRed protocol for HRP

\*consider early block with 0.25% H<sub>2</sub>O<sub>2</sub> for 5-10min