

## 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