

Embryo's

- Less than E12 Fix 2% PFA 1-2hrs
 - E12 or older Fix 2% PFA 2-4hrs
 - then vibrotome 300micron sections
- 1) Blocking buffer 5% Donkey Serum/0.3% Triton/ 1XPBS, block for 1-2hrs
 - 2) 1° antibody: 1:100 VE-Cad (BV13/4) Imclone monoclonal rat anti-mouse in 1X blocking buffer
 - 3) Incubate overnight humidified chamber at 4°C
 - 4) PBS wash 5min x 3
 - 5) 1:200 Donkey anti-rat Cyn3 in 1X BB
 - 6) Incubate RT x 2hrs
 - 7) PBS wash 10min x 3
 - 8) Vectashield mount