

## Whole Mount Retina IHC

### Day 1

1. Fix eyes in **4% PFA** for 45-60'
2. Was 3 x 10' in PBS
3. Dissect retinas
4. Block with **1% BSA/0.5% Tween 20** in PBS for 30' at rt
5. Wash 2x in **PBlec** (0.1 nM CaCl<sub>2</sub>, 0.1 MgCl<sub>2</sub> mM, 0.1 MnCl<sub>2</sub> mM, and 1% Triton X-100 in PBS), incubate in last wash at least 30'.
- 6a. Incubate 500 ml o/n at 4°C with **Isolectin B4-biotin (1:10)** (SIGMA, L-2140) in PBlec, shaking.
- 6b. Incubate 500 ml o/n at 4°C with Lectim-FITIC 1:50 (Sigma 107K4118) in PBlec, shaking. 2 minutes maximum velocity RT.

### Day 2

1. Wash 5 x 10' in PBS at rt
2. Incubate o/n at 4°C with **Streptavidin-Alexa 488 (1:100)** centrifuge before use (Molecular Probes, S11223) and **DAPI (1:1000)** (Invitrogen, D3571) in 0.5% BSA/0.25% Tween 20 in PBS, shaking

### Day 3

1. Wash 5 x 10' in PBS at rt
2. Mount in **ProLong Gold anti-fade** (Invitrogen, P36934)

**HAMSTER ANTI MOUSE CD31  
MONOCLONAL ANTIBODY**

**CATALOG NUMBER:** MAB1398Z

**LOT NUMBER:** xxxxxx

**QUANTITY:** 100 µg

**CONCENTRATION:** 1 mg/mL

**SPECIFICITY:** Reacts with the 130-140 kDa protein, PECAM-1 found on mouse monocytes, platelets, granulocytes and endothelial cells. 2H8 effectively inhibits transmigration of activated polymorphonuclear cells and monocytes across the endothelium. In a mouse model for acute peritonitis - 2H8 blocks acute inflammation.

**ISOTYPE:** IgG

**CLONE NAME :** 2H8

**APPLICATIONS:** Flow Cytometry: 0.1 µg/mL  
Blocking  
Optimal working dilutions must be determined by end user.

**FORMAT:** Purified immunoglobulin - Ig fraction

**PRESENTATION:** Liquid in phosphate buffered saline containing no preservatives

**STORAGE/HANDLING:** Store at -20°C only. Avoid repeated freeze-thaw cycles.

**REFERENCES:**

1. Yong, X et al. (1993). *Proc. Natl. Acad. Sci.* **90**: 5569-5573
2. Bogen, S. et al. (1994). *J. Exp. Med.* **179**: 1059-1064.
3. Newman, P.J. et al. (1992). *J. Cell. Biol.* **119**: 239-246.

*For research use only; not for use as a diagnostic.*