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$_2$, 0.1 MgCl$_2$ mM, 0.1 MnCl$_2$ 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 polymophonuclear 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.

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