

Immunohistochemistry for detecting zTie2

Masahiro Shin (07/05/2019)

fix 4%PFA RT 1hr (4°C O/N reduces staining intensity)

3%H₂O₂ treatment RT 1hr

store in 100%MetOH before use

<1st day>

wash with PBSTw/0.1%TritonX100

ProK(30μg/mL)/PBSTw 2min RT for 36hpf embryos

8%PFA/PBS 30min RT (note: Tie2 staining works without ProK step albeit with the weaker staining)

wash with PBSTw

blocking with PBSTw/2%blocking reagent (Rhoce) RT 2-3hr

1/100 zTie2 antibody (#AF928, R&D systems) in blocking solution 4deg O/N

<2nd day>

wash with PBSTw 30min RT x6

1/5000 rabbit anti goat IgG-HRP (#81-1620, Invitrogen) in blocking solution

<3rd day>

wash with PBSTw RT x6

wash with PBS

TSA-Cy3 reaction RT 3hr

wash with PBSTw/0.1%TritonX100 RT O/N