

PH3/4D5 Ab:

- Fix 4% PFA 4 degrees C O/N.
 - Wash in PBT, dechorionate, wash
 - Incubate in block O/N 4 degrees C. Block = 2% NGS, 2% BSA, 1% DMSO, 0.5% triton X, in PBT.
 - 1:800 Antibody in block O/N 4 degrees C.
 - wash out 2 hours in block.
 - 1:500 Alexa Fluor goat anti-rabbit secondary antibody 4 degrees C O/N.
 - counterstain with phalloidin-TR
- (4D5- use 1:100 ab and goat anti-mouse secondary)

HuC HRP

- fix embryos, wash, etc
- quench in 100uL H2O2/1mL PBT 1 hour, PBT wash
- block 5% NGS several hours
- 1:500 HuC Ab in block 4 degrees O/N
- wash out in PBT, add 1:500 goat anti mouse HRP in block 4 degrees O/N
- wash out, stain with DAB kit, fix 4 degrees O/N

a6F and other cell membrane labels

- fix in 80% MeOH/20% DMSO 2 hours RT
- PBT wash, block 10% NGS/PBT several hours
- 1:100 Ab in block 4 degrees O/N
- wash out, add 1:500 goat anti mouse secondary
- add phalloidin 4 degrees C O/N

Nok Ab

- fix in 4% PFA 3 hrs RT, block is 2% NGS, 1% DMSO, 1:500 rabbit anti-Nok

Acetylated Tubulin Antibody Protocol

- 1) Fix (usually at 24hpf) 1-2 hrs rm temp (max time) 4% paraformaldehyde
- 2) Wash 3 x PBT (30 minutes total) and dechorionate
- 3) Wash (permeabilization step):
 - 2 x 5 min dH2O
 - 10 sec 1mg/mL trypsin in H2O
 - dH2O 2x fast
 - PBT 1 min
 - PBT 5 min 2x
- 4) incubate 3 hrs PBT + (1% BSA) + 2% NGS + 1% DMSO rm temp

- 5) dissect brain during incubation if desired
- 6) incubate in fresh blocking sol'n and 1:1000 acetylated tubulin Ab O/N 4 degrees up to one week
- 7) wash with PBT several hours RT, several washes
- 8) add secondary Ab O/N in blocking sol'n 1:500 (goat anti-mouse IgG Alexa Fluor 488)
- 9) wash out in PBT
- 10) mount for confocal imaging