Zebrafish Immunohistochemistry

Protocol from:
Aljiboury, A. A., Mujcic, A., Cammerino, T., Rathbun, L. I., & Hehnly, H. (2021). Imaging the early zebrafish embryo centrosomes following injection of small-molecule inhibitors to understand spindle formation. *STAR Protocols*, *2*(1). https://doi.org/10.1016/j.xpro.2020.100293

1. Breed fish and collect embryos. Raise in incubator until developmental stage of interest.

2. Fix with 4% paraformaldehyde (PFA) + 0.5% Triton-X (T-X).
    a. For older embryos: overnight at 4°C
    b. For younger embryos: fix at room temperature (RT) without detergent for 2-4 hours, then overnight at 4°C

3. After fixation, remove fix and wash 3x in PBST 2 (1X Phosphate Buffered Saline (PBS) + 0.1% Tween-20)

4. Dechorionate embryos in PBST 2.

5. Incubate embryos in wash solution for 1 hour at RT (blocking step).

6. Incubate embryos in primary antibody diluted in wash solution (typically between 1:200-1:1000 dilution)
    a. Option 1: incubate in primary overnight at 4°C
    b. Option 2: incubate in primary for 4 hours at RT.
    c. Diluted primary antibodies can be reused 1-2 times.

7. Remove primary antibody solution and wash embryos 5x with wash solution. Washes should be 5-10 minutes each.
8. Incubate embryos in wash solution for 30-60 min at RT (blocking step).

9. Incubate embryos in secondary antibody (Jackson Immuno Research Labs, Invitrogen or Molecular probes) diluted in wash solution (typically between 1:200-1:1000 dilution).
    a. Option 1: incubate in secondary overnight at 4°C. NOTE: can only be used if primary was also incubated overnight.
    b. Option 2: incubate in secondary for 3-4 hours at RT.

10. Remove secondary antibody solution and wash embryos 5X with wash solution. Washes should be 5-10 minutes each.

11. If necessary, incubate embryos in DAPI (Sigma-Aldrich cat. D9542-10MG) diluted in PBS (1:1000) for 1.5 hours at RT, or use actin dropper at 1 drop per mL for 1 hr at RT.

12. Wash embryos 3x with PBS.

13. Agar. (Thermo Fisher Scientific, cat. 16520100) mount or hard mount with Prolong (Fisher Scientific, P36934) before imaging on microscope.

Solutions:

Fix:
Make 4% paraformaldehyde in PBS by heating with stir plate until PFA has incorporated fully and solution boils. Keep Kimwipes in the top of flask to minimize evaporation. Add Triton-X to PFA solution to 0.5% Triton-X concentration.

PBST 2 (Filter Sterilize):
0.1% Tween-20 in PBS (phosphate buffered saline)

Wash solution (in 1X PBS) (Filter Sterilize):
1% DMSO (Fisher cat. BP231 100)
1% BSA (Fisher cat. BP1600-100)
0.1% Triton-X (T-X) (Fisher cat. BP151500)