

Antibody Staining Protocol

Optimized for *Aurelia* jellyfish, successfully used on various cnidarians and ctenophores

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Primary Antibodies	Secondary/Additional Antibodies

Fixation

- 1. Anesthetize animals in 7.3% MgCl₂ or 0.8mM menthol in artificial sea water (ASW)
- 2. Fix animals in 4% formaldehyde for 1 hour at room temperature (RT)
- 3. Wash in PBSTr 4x. Can keep at 4°C for short-term storage.

Day 1

- 4. Wash 4x in PBSTr over a 2-hour period at RT: (1) (2) (3) (4)
- 5. Block in 3% Normal Goat Serum (NGS) for 1 hour @ RT (or overnight @4C)
- 6. Add primary antibodies, and leave on rotator overnight at 4°C (or 2 hours RT)

Day 2

- 7. Wash 4x in PBSTr over a 2-hour period at RT: (1) (2) (3) (4)
- 8. Block in 3% NGS for 1 hour at RT (or overnight at 4°C)
- 9. Add secondary antibodies, and leave on rotator overnight at 4°C (or 2 hours at RT)

Day 3

- 10. Wash 4x in PBSTr over a 2-hour period at RT
- 11. Optional: Add fluorescent stains (e.g. Phalloidin, Topro)
- 12. Mount samples on slides

Common Antibodies and Stains

Anti-acetylated tubulin (A): mouse, 1:1000
Anti-tyrosinated tubulin (T): mouse, 1:800
Anti-GnRH (G): rabbit, 1:1000
All Secondary Alexa Antibodies: 1:200

Anti-FMRF (F): rabbit, 1:500
Anti-Ncol1 (N): rabbit, 1:1000
Anti-ELAV (E): rabbit, 1:200

Phalloidin (red): 1:20, incubate for 1 hr

Topro (blue): 1:1000, incubate for 15 min