

Cell Labeling with EdU

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

Last Updated 8/11/17

Document created by David Gold (www.DavidAdlerGold.com)

1. Incubate animals in 10mM EdU for desired length of time

Fixation

2. Anesthetize animals in 7.3% MgCl₂ or 0.8mM menthol in artificial sea water (ASW)

3. Fix animals in 4% formaldehyde for 30-minutes at room temperature (RT)

4. Wash 4x in PBSTr over a 30-minute period at RT: (1) (2) (3) (4)

5. Block in 3% Normal Goat Serum (NGS) for 1 hour @ RT (or overnight @4C)

EdU Reaction

6. Prepare cocktail reaction by combining the following reagents **in order**:

- 176 µl PBS
- 2 µl copper sulfate (EdU box in fridge)
- 0.4 µl Alexa Fluor azide (Mike's -20 fridge)
- 20 µl **freshly made** ascorbic acid
 - 400mg ascorbic acid in 2 mL of DI water

8. Remove liquid from samples and replace with 200 ul EdU cocktail to samples

- Make sure to use the cocktail within 15 minutes of preparation

9. Incubate in cocktail for 30 minutes

10. Wash 4x in PBSTr at RT: (1) (2) (3) (4)

11. Optional: Proceed to antibody staining and/or Add fluorescent stains

- **Note:** *Phalloidin is incompatible with EdU*

EdU Cocktail Calculator

Reagent	Amount per sample	Number of samples	Final Amount
PBS	176 µl	x _____	_____
Copper sulfate (CuSO ₄)	2 µl	x _____	_____
Alexa Fluor azide	0.4 µl	x _____	_____
ascorbic acid	20 µl	x _____	_____