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Immunofluorescence Staining of Sea Urchin Embryos V.1

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David Booth[1]

[1]University of California, San Francisco

David Booth
University of California, San Francisco

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We use this protocol and it's working

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Abstract

In this lab, you will use immunofluroescence stainging to visualize the wondrous cellular transformations that occur throughout sea urchin development. The goals of this module are
1. To learn about marine organisms at Marine Resources and some of their biology
2. Understand the steps of immunofluorescence and its relative advantages
3. Visualize stained embryos and understained the basic tradeoffs of some types of imaging techniques

Guidelines

Handle embryos with care
Embryos are fragile after crosslinking. Quick pipetting generates a lot of shear that can disintegrate embryos and risks the possibility of sucking-up and discarding embryos. Therefore, be patient with pipetting to get the best staining.

Materials

Filtered Sea Water

Formaldeyde

Phosphate Buffered Saline (PBS)

PBS + 0.05% (v/v) Tween-20 (PBST)

PBST + 4% Normal Goat Serum (Blocking Solution)

PBS + 50% (v/v) Glycerol

Antibodies
- Primary antibody: Mouse anti-Tubulin
- Secondary antibody: Donkey anti-mouse IgG conjugated to Cy3

Dyes
- Filamentous actin: Phalloidin conjugated to AlexaFluor488 (
- DNA: DAPI

Safety warnings

Formaldehyde
Formaldehyde is a very active crosslinker and can crosslink your tissue. Handle with care!

Fix Cells

1 **Prepare cells for fixation**
- Place 300 µl of embryos in filtered seawater in 1 well of a 96-well, round-bottom dish.

2 **Fix cells with paraformaldeyde**
- Add 100 µl of 16% (w/v) paraformaldehydle to 300 µl of embryos in seawater
- Gently rock cells for 1 h at room temperature

3 **Rinse embryos in filtered seawater**
- Take embryos from the rocker and let them settle to the bottom of this dish for ~2 min
- Gently remove 250 µl of liquid from the well by pipetting from one side of the dish, just below the meniscus
- Gently pipette 200 µl of filtered seawater down one side of the dish to gently cover the embryos
- Let embryos settle to the bottom and then gently emove the liquid.
- Repeat for a total of 4 rinses.
- Leave the embryos in 150µl of liquid to proceed to the next step.

Block

4 **Rinse embryos into PBST with 4% Normal Goat Serum (Block Solution)**
- Gently add 200 µl of block solution to the embryos
- Gently remove 200 µl of block solution the embryos
- Repeat for a total of 2 rinses

5 **Permeabilize and Block embryos in PBST with 4% Normal Goat Serum (Block Solution)**
- Gently add 200 µl of block solution to the embryos
- Incubate for 1 h at room temperature with gentle rocking

Add Primary Antibody

6 **Prepare primary antibody**
- Dilute mouse anti-Tubulin antibody 1:500 in Block solution

! Remember that you will need 200 µl of primary antibody per well

7 **Add primary antibody to embryos**
- Gently remove 200 µl of block solution from the embryos
- Gently add 200 µl of primary antibody to the embryos
- Incubate for overnight at room temperature with gentle rocking

8 **Rinse away primary antibody**

- Gently remove 200 µl of primary from the embryos
- Gently add 200 µl of block solution to the embryos
- Incubate for 5 min at room temperature
- Repeat rinses for a total of 4 rinses.

Add Secondary Antibody and Dyes

9 **Prepare secondary antibody**

- Dilute donkey anti-mouse antibody conjugated to AlexaFluor568 1:500 in Block solution
- In the same solution with the secondary antibody, dilute phalloidin conjugated to AlexFluor488 1:100 and DAPI 1:1,000

! Remember that you will need 200 µl of secondary antibody per well

! Remember that dyes are light sensitive, so keep them covered prior to use

10 **Add secondary antibody to embryos**

- Gently remove 200 µl of block solution from the embryos
- Gently add 200 µl of secondary antibody/dyes to the embryos
- Incubate for 1 h at room temperature and with gentle rocking

! Cover the samples to protect dyes from photobleaching.

11 **Rinse away dyes**

- Gently remove 200 µl of secondary antibody/dyes from the embryos
- Gently add 200 µl of block solution to the embryos
- Incubate for 10 min at room temperature
- Repeat rinses for a total of 3 rinses.

12 **Exchange into PBS+50% Glycerol**

- Gently remove 200 µl of block solution from the embryos
- Gently add 200 µl of PBS+Glycerol to the embryos
- Incubate for 5 min at room temperature
- Repeat rinses for a total of 3 rinses.
- Gently 200 µl of liquid from the embryos to prepare for the next step.

Mounting

13 **Place embryos in a glass bottom dish**

- Fetch a glass bottom dish and glass cover slip
- Make a wide bore pipette tip by cutting off the bottom of a 200 µl pipette tip with a razor blade scissors
- Gently pipette embryos up and down with the wide bore tip and gently transfer ALL of the liquid from the well to the glass bottom dish
- Gingerly place a cover slip on top of the drop of liquid without incorporate bubbles by first letting one edge of the cover clip touch the dish and then slowly lowering the other side into the dish

! Keep the samples in the dark until imaging on a microscope