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Jun 13, 2022

Immunofluorescence Staining of Sea Urchin Embryos V.2

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dx.doi.org/10.17504/protocols.io.5jyl8no8rl2w/v2

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In this lab, you will use immunofluroescence staining to visualize the wondrous cellular transformations that occur throughout sea urchin development. The goals of this module:

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 embryos stained with antibodies and vital dyes with basic imaging techniques

DOI

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David Booth 2022. Immunofluorescence Staining of Sea Urchin Embryos. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8no8rl2w/v2 David Booth

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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.

Filtered Sea Water

16% (w/v) Formaldeyde

Phosphate Buffered Saline (PBS)

PBS + 0.1% (v/v) Triton X-100 + 0.05% (v/v) Tween-20 (PBST)

PBST + 4% Normal Goat Serum (Blocking Solution)

PBS + 60% (v/v) Glycerol

Antibodies

• Primary antibody: Mouse anti-Tubulin (DM1A)
• Secondary nanobody: Alpaca anti-mouse IgG conjugated to AlexaFluor568

Dyes

• Filamentous actin: Phalloidin conjugated to AlexaFluor488
• DNA: DAPI or Hoechst

Formaldehyde

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

Fix Cells

1 Prepare cells for fixation

• Place 225 µl of embryos in filtered seawater in 1 well of a 96-well, round-bottom dish.

2 Fix cells with paraformaldeyde

• Add 75 µl of 16% (w/v) paraformaldehydle to 225 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 200 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

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• Let embryos settle to the bottom and then gently remove the liquid.
• Repeat for a total of 2 rinses.
• Leave the embryos in 100µl 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 (DM1A) 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 in the cold room with gentle rocking

Add Secondary Antibody and Dyes

8 Prepare secondary antibody

• Dilute Alpaca anti-mouse nanobody conjugated to AlexaFluor568 1:500 in TBST
• In the same solution as the nanobody, dilute phalloidin conjugated to AlexFluor488 1:50 and DAPI 1:500
! Remember that you will need 50 µl of secondary antibody per well
! Remember that dyes are light sensitive, so keep them covered prior to use

9 Add secondary antibody to embryos

• Gently remove 100 µl of block solution from the embryos
• Gently add 100 µl of secondary antibody/dyes to the embryos
• Incubate for 1-2 h at room temperature and with gentle rocking
! Cover the samples to protect dyes from photobleaching.

10 Rinse away dyes

• Gently remove 200 µl of secondary antibody/dyes from the embryos
• Gently add 200 µl of PBST to the embryos
• Incubate for 10 min at room temperature
• Repeat rinse for a total of 2 rinses.

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• Gently remove 200 µl of PBST from the embryos
• Gently add 200 µl of PBS to the embryos
• Incubate for 10 min at room temperature
• Repeat rinse for a total of 2 rinses.

11 **Exchange into PBS+60% 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
• Spin at 100xg for 30 sec to settle the embryos to the bottom.
• Gently remove 200 µl of of liquid from the embryos
• Gently add 200 µl of PBS+Glycerol to the embryos and leave

Mounting

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

• Fetch a glass bottom dish and glass cover slip or chamber slide
• Make a wide bore pipette tip by cutting off the bottom of a 1000 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 slip touch the dish and then slowly lowering the other side into the dish
! Keep the samples in the dark until imaging on a microscope

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