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Protocol

Immunostaining of *Ectocarpus* Cells

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This article describes an immunostaining protocol for *Ectocarpus* that was optimized for the detection of tubulin but could be used with any suitable antibody. *Ectocarpus* has small but relatively transparent cells and the uniseriate filaments can be grown directly attached to the surface of microscope slides. These features make *Ectocarpus* particularly suitable for high resolution imaging approaches, both in vivo or after fixation. All incubations described below are carried out on a platform shaker at room temperature. Use high-quality microscope slides to avoid imperfections in the glass that can be a problem for confocal laserscan microscopy analysis.

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Antibodies appropriate for experiment (primary and secondary; see Steps 6 and 7)

BABB <R>

Blocking solution (2% [w/v] nonfat dry milk, prepared fresh just before use in mPBS)

Ectocarpus filaments attached to high-quality microscope slides

Liquid nitrogen

Methanol (100%)

mPBS <R>

$NaBH_4$ (100 mM, prepared in mPBS)

Nail polish (e.g., Sally Hansen's clear "Hard as Nails")

PHEM <R>

Solution C <R>

Solution C plus enzymes (add 175 mg celF and 1 g hemicellulase to 25 mL of Solution C just before use)

Triton X-100 (5%, prepared in mPBS)

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Equipment

Cardboard box (see Step 6)

Coverslips

Microscope slides (with high quality glass; e.g., Fisher 12-550-42)

Paper towels, wet (see Step 6)

Parafilm

Petri dishes (90 and 140 mm)

Plastic film (e.g., Saran wrap)

METHOD

1. Dip slides with attached *Ectocarpus* material rapidly into liquid nitrogen (immerse for <1 sec) and immediately immerse in PHEM solution in a fresh 35 × 10-mm Petri dish for 1 h (or overnight at 4°C).
2. Remove the PHEM, rinse three times with mPBS (5 min each time), and immerse in 5% Triton X-100 in mPBS. Incubate overnight at room temperature.
3. Rinse three times with mPBS and then immerse in 100 mM $NaBH_4$. Incubate for 4 h or until the solution stops bubbling (indicating that the reducing reaction is complete).
4. Rinse three times with mPBS. Immerse in Solution C, then in Solution C plus enzymes. Incubate for 1 h.
5. Rinse three times with mPBS. Immerse in blocking solution and incubate overnight at room temperature.
6. Rinse three times with mPBS. Line the bottom of a 90-mm Petri dish with Parafilm. Pipette ~20 µL of the primary antibody onto the Parafilm then place the slide face-down on top of the drop, so that the filaments stuck to the slide are in contact with the antibody. Place the 90-mm Petri dish in a 140-mm Petri dish (or in a glass crystalizer) with ~1/2 in of wet paper towels on the bottom. Cover the large dish with plastic film (e.g., Saran wrap) and put into a light-proof cardboard box. (This keeps the chamber humid so that the filaments do not dry out.) Incubate for 4 h or overnight.
7. Rinse three times with mPBS and add the secondary antibody. Incubate for 4 h or overnight as described in Step 6. (Exclusion of light is particularly important for the secondary antibodies.)

   If you want to carry out a double labeling, label with the first pair of antibodies (monoclonal primary, then secondary) as above, then block with blocking solution overnight before labeling with the second pair of antibodies (polyclonal primary, then secondary).
8. Rinse three times with mPBS and then three times with 100% methanol at −20°C. Note that methanol dries very quickly—do not let the cells dry out.
9. Mount by pipetting ~20 µL of BABB onto the microscope slide, and then rapidly placing a coverslip on the slide. Touch the edge of the slide on a paper towel to remove excess methanol. Wait for ~20 sec, and then paint around the perimeter of the coverslip with nail polish. Wait for 1 h and then repeat the sealing with the nail polish to eliminate small holes that could cause the material to dry out.

RELATED INFORMATION

For further details regarding the biology of *Ectocarpus* and the development of genomic and genetic tools for this organism, see ***Ectocarpus*: A Model Organism for the Brown Algae** (Coelho et al. 2012).

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RECIPES

BABB

Benzyl alcohol
Benzyl benzoate

Mix benzyl alcohol:benzyl benzoate at a ratio of 1:2.

mPBS

Reagent	Quantity (for 3.2 L)	Final concentration
NaCl (MW = 58.44)	25.6 g	136 mM
KCl (MW = 74.55)	0.64 g (or 8.64 mL of 1 M stock)	2.7 mM
KH_2PO_4 (MW = 136.09)	0.7408 g	1.7 mM
Na_2HPO_4 (MW = 141.96)	3.648 g	8.03 mM
Glycerol (MW = 92.10) (density = 1.261 g/cm^3)	160 mL	685 mM
Sodium azide (MW = 65.02)	3.2 g	15.4 mM
BSA (MW = 67.000)	3.2 g	0.015 mM

The pH should be 7 or slightly above. Adjust the volume to 3.2 L with distilled water. This solution is stable and can be used for several weeks.

PHEM

Reagent	Quantity (for 200 mL)	Final concentration
PIPES	3.68 g	60 mM
HEPES	1.19 g	21 mM
EGTA	0.76 g	10 mM
$MgCl_2$ (1 M)	400 µL	2 mM
NaCl	8 g	685 mM

Dissolve the reagents in distilled water and adjust the pH to 7.5 with KOH. Adjust the volume to 179 mL with distilled water. Add 1 mL of 25% glutaraldehyde and 20 mL of 32% paraformaldehyde. This solution works best if it is made fresh just before use.

Solution C

Reagent	Quantity (for 1 L)	Final concentration
NaCl (MW = 58.44)	5.8 g	100 mM
$MgCl_2$	4.1 g	20 mM
KCl (MW = 74.55)	0.15 g	2 mM
BSA (MW = 67,000)	2 g	29.8 µM
2-(*N*-morpholino) ethanesulfonic acid (MES) (MW = 195.2)	2.13 g	0.01 mM
Sorbitol (MW = 182.17)	154.85 g	850 mM
EGTA (MW = 292.24)	0.38 g	1 mM

Adjust the pH to 5.8 with Tris base. Adjust the volume to 1 L with distilled water. Store in 50-mL aliquots at −20°C.

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REFERENCE

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