Protein Extraction from Freshly Isolated Algae

Protocol author: Virginia Weis
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Reagents

Filtered Seawater

Triton X-100

Extraction buffer: pH 7.4
100 mM Tris
10 mM EDTA
100 mM NaCl
The day of use, add 1 aliquot of Protease inhibitor cocktail (PIC) to 10 ml of buffer

Acid washed glass beads (400-600 um).

Procedure

A. Isolation of algal pellet from whole animals

1. Obtain 25 frozen *A. elegantissima*
2. Partially defrost and cut off pedal discs with a razor blade.
3. Grind animals in mini-food processor in 30 ml FSW.
4. Divide into 4 50 ml tubes, rinse processor and include rinsate.
5. Spin 6 min at 2500 g
6. Rinse and re-spin approximately 5 times. Vigorously resuspend pellet each time.
7. Filter each tube of algae through 2 layers of cheesecloth to remove large chunks of tissue
8. This procedure should yield about 6 ml of algal pellet.

B. Extraction of algal protein homogenate

1. Work with 1.5 ml of above algal pellet. Freeze the remainder
2. To this 1.5 ml, add 10 ml of FSW with 2% triton. Resuspend algae.
3. Spin at 2,500 g for 6 min. Supernatant should have greenish-yellow tint. Pour off supernatant
4. Rinse pellet once in FSW and respin.
5. Pour off supernatant and add about 3.75 ml of extraction buffer (with PIC). Resuspend algae and place suspension in a **glass** culture tube.

6. Add 1-2 ml of glass beads (acid washed).
7. Vortex suspension for 30sec and then place on ice for 30 sec.
8. Repeat vortex and icing a total of 20 times
9. Pipette out suspension, away from glass beads and place in microfuge tubes.
10. Spin at 15,000 rpm in microfuge for 5 min. Resulting supernatant should be a deep, clear orange.
11. Determine protein concentration with Bradford assay.