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Working

Protein Extraction of Symbiodiniaceae freshly isolated from Anthopleura elegantissima

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Weis Lab Oregon State | Anthopleura-Microbe Model System

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ABSTRACT

PROTOCOL STATUS

Working

We use this protocol in our group and it is working.

SAFETY WARNINGS

BEFORE STARTING

Get ice.

Mix extraction buffer

1 Recipe (adjust volumes as needed):
50 mL - 100 mM Tris
50 mL - 10 mM EDTA
50 mL - 100 mM NaCl

Adjust pH of the buffer to 7.4.

The day of use, add 1 aliquot of Protease Inhibitor Cocktail (PIC) to 10 mL of buffer.

A few options for PIC:

1. https://www.sigmaaldrich.com/catalog/product/sigma/p9599?lang=en®ion=US

P9599 from Sigma-Aldrich (extraction from plant tissue)

2. https://www.sigmaaldrich.com/catalog/product/sigma/s8820?lang=en®ion=US

S8820 from Sigma-Aldrich (general use)

Gather materials

2 1. 2% Triton X100 in FSW
2. FSW
3. Acid washed glass beads (400-600µm in diameter): https://www.sigmaaldrich.com/catalog/product/sigma/g8772?lang=en®ion=US
G8772 from Sigma-Aldrich

3 If you are working with an algae culture, skip to step 12.

Isolation of algal pellet from whole animal

Isolation of algal pellet from whole animal

4 Obtain 25 frozen small *Anthopleura elegantissima* (You could use 10 medium or 3 large animals also). Adjust the number of animals as needed.

5 Partially defrost and cut off pedal discs with a razor blade (keep the algal-rich tentacle crown).

6 Grind animals in mini-food processor or glass-teflon grinder in 30 ml FSW.

7 Divide into 4-50 ml tubes, rinse processor and include rinsate.

8 Spin for 6 min at 2500 xg at 4˚C.

9 Rinse and re-spin approximately 5 times. Vigorously resuspend pellet via vortex each time.

10 Filter each tube of algae through 2 layers of cheesecloth to remove large chunks of tissue

11 This procedure should yield about 6 ml of algal pellet for *A. elegantissima*.

Extraction of algal protein

12 Work with 1.5 ml of above algal pellet. Freeze the remainder.

13 To this 1.5 ml, add 10 ml of FSW with 2% triton X100. Resuspend algae via vortex.

14 Spin at 2,500 xg for 6 min at 4˚C. Supernatant should have greenish-yellow tint. Remove and discard supernatant.

15 Rinse pellet once with 10 ml of FSW with 2% triton X100 and spin again at 2,500 xg for 6 min at 4˚C.

16 Remove and discard supernatant and add 3.75 ml of extraction buffer (with PIC). Resuspend algae and place suspension in a **glass** culture tube.

17 Add 1-2 ml of glass beads (acid washed).

18 Vortex suspension for 30 sec and then place on ice for 30 sec.

19 Repeat vortex and icing a total of 20 times

20 Remove suspension, away from glass beads and place in microfuge tubes.

21 Spin at 15,000 rpm in microfuge for 5 min at 4˚C. Resulting supernatant should be a deep, clear orange.

22 Determine protein concentration with Bradford or other protein quantification assay.

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