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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





ABSTRACT

PROTOCOL STATUS

Working

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

SAFETY WARNINGS

BEFORE STARTING

Get ice.

Mix extraction buffer

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:

 $1. \ \underline{https://www.sigmaaldrich.com/catalog/product/sigma/p9599?lang=en@ion=US}$

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)

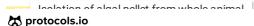
Gather materials

1.2% Triton X100 in FSW

2. FSW

 $3. \ Acid \ washed \ glass \ beads \ (400\text{-}600\mu\text{m in diameter}): \\ \underline{\text{https://www.sigmaaldrich.com/catalog/product/sigma/g8772?lang=en@ion=US}}$ G8772 from Sigma-Aldrich

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



| 15 | isolation of algal peliet from whole animal | |
|-----------------------------|--|--|
| 4 | Obtain 25 frozen small <i>Anthopleura elegantissima</i> (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 <i>A. elegantissima</i> . | |
| 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.
- $21 \qquad \text{Spin at 15,000 rpm in microfuge for 5 min at 4°C. Resulting supernatant should be a deep, clear orange.}$
- $22 \qquad \hbox{Determine protein concentration with Bradford or other protein quantification assay}.$

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