Protein extraction from Aiptasia

OSU Weis Lab

Abstract

This protocol, developed in 2011 by Angela Poole while in the Weis lab, is a quick prep for protein extraction from Aiptasia adults.

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Before start

RIPA extraction buffer:

- 100mM Tris, pH 7.4
- 100mM NaCl
- 10mM EDTA, pH 8.0

*Bring to desired volume with Nanopure water and just before use, dissolve one cOmplete™, mini, protease inhibitor tablet in 10mL of RIPA Buffer. Be sure to let the tablet dissolve on its own- do not vortex!!

Materials

cOmplete™, Mini Protease Inhibitor Cocktail 11836153001 by Roche

Protocol

Step 1.

Prepare anemones by placing in the incubator in artificial seawater (ASW) 3-4 days prior to extraction. Be sure to change out the (ASW) each day to remove any residual debris.

Step 2.

Transfer 4-5 large anemones to a small tissue grinder on ice with 0.5-1 mL of chilled extraction buffer. Upon removal from their original container, anemones should be blotted on Kimwipes/weigh paper to remove as much water as possible.

Step 3.

Transfer homogenate to a 1.5 mL tube and centrifuge at 14,000xg for 15 minutes at 4°C. This step will pellet the dinoflagellates and cell debris, while the protein will be in the supernatant.

Step 4.

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Remove the supernatant (be careful not to disturb the white lipid layer!) and place in a new tube.

Step 5.
Determine the concentration of your protein (we used the Bradford assay).

Step 6.
Aliquot your protein into 100 µl volumes and store in the -80°C freezer until further use. Freeze-thaw cycles should be avoided and protein can be kept for a short period in the fridge on ice.

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