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General Total Protein Sample Preparation Protocol for the Immunodetection of Auxenochlorella protothecoides Proteins.

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Dimitrios Camacho[1], Sabeeha S. Merchant[1]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA

Merchant Lab UC Berkeley

Dimitrios Camacho
University of California, Berkeley

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We use this protocol and it's working

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Abstract

This protocol describes a general method for quickly preparing and storing protein samples for the immunodetection of *Auxenochlorella protothecoides* proteins. The protocol was developed for metal free applications where the metal contents of *Auxenochlorella protothecoides* cells are of importance to the proteins studied. This protocol should be adapted to optimize sampling conditions for each protein of interest.

Guidelines

This protocol should be tailored to your specific protein of interest. For example, if you are also interested in light responsive proteins, you may wish to extract proteins in a specific light regime.

Use only ICPMS grade trace metal free Ultra-pure ICP-MS grade Milli-Q H_2O.

Review the certificates of analysis for each chemical used to verify potential metal contamination concentrations are minimized.

Materials

1. Metal free 15 mL tubes - Globe Scientific Inc. Centrifuge, high performance, red screw cap, assembled, polypropylene. Cat. No. 6295, with a maximum rating of 17,000 ×*g.*
2. Metal free 50 mL tubes - Globe Scientific Inc. Centrifuge, high performance, red screw cap, assembled, polypropylene. Cat. No. 6297, with a maximum rating of 20,000 ×*g.*
3. 1 L HDPE bottle, 4 bottles.
4. Ultra-pure 6 M HCl.
5. Ultra-pure ICP-MS grade Milli-Q H_2O.
6. Trace metal grade Na_2HPO_4 anhydrous (dibasic, MW = 138 g/mol).
7. Trace metal grade $NaH_2PO_4 \cdot H_2O$ (monobasic, MW = 138 g/ mol).
8. cOmplete™ ULTRA Tablets, Mini, EASYpack Protease Inhibitor Cocktail.
9. 1-10 L dewar of liquid nitrogen.
10. 1.5 mL metal free screw cap tubes with gasket.
11. 1.5 mL metal free conical tubes.
12. Acid washed, metal free glass beads 425-600 µm.
13. Acid washed, metal free glass beads 4 mm.
14. Centrifuge.
15. Sterile hood.
16. Biospec Mini-BeadBeater-16
17. Liquid nitrogen flash freezing tube rack.
18. RAININ P100, P1000 pipettes and tips.

Safety warnings

Liquid nitrogen can displace oxygen and cause asphyxiation. If transporting greater than 10 L of liquid nitrogen, do not accompany the dewar in the elevator. Have someone take the dewar out of the elevator at your floor. Do not cap tubes tightly after flash freezing in liquid nitrogen. Tubes may explode if capped when they warm up. Do not use nitrile or rubber gloves to handle liquid nitrogen. Use cryogenic gloves, closed toe shoes, a lab coat, a cryogenic apron, and a face shield when handling liquid nitrogen.

Before start

1. Wipe down all work surfaces with 70% EtOH.

2. Prepare a bucket of wet ice. Add water to the ice so that the tubes will be in contact with the ice water 0 °C .

2. Fill a 1-10 L dewar of liquid nitrogen.

3. Prepare cell lysis tubes.

3.1. Add 200 mg of 425-600 µm acid washed glass beads to a 1.5 mL screw cap tube with gaskets.

3.2. Add one 4 mm glass bead to the tube. Keep the tubes on wet ice.

4. Make the trace metal grade [M] 10 millimolar (mM) sodium-phosphate solution, pH 7 and protease inhibitor cocktail mixture.

4.1. Acid wash the 1 L HDPE bottles (Quinn and Merchant, 1998),(Camacho and Merchant, 2024).

4.2. Make [M] 1 Molarity (M) NaH_2PO_4 by adding 138 g of $NaH_2PO_4 \cdot H_2O$ to a 1 L bottle with stirring and fill to 1 L with Milli-Q H_2O. Store at 4 °C.

4.3. Make [M] 1 Molarity (M) Na_2HPO_4 by adding 142 g of Na_2HPO_4 (anhydrous) to a 1 L bottle with stirring and fill to 1 L with Milli-Q H_2O. Store at 4 °C.

4.4. Make [M] 1 Molarity (M) sodium-hosphate solution, pH 7 by mixing 390 mL of [M] 1 Molarity (M) NaH_2PO_4 and 610 mL of [M] 1 Molarity (M) Na_2HPO_4. Store at 4 °C.

4.5. Dilute [M] 1 Molarity (M) sodium-phosphate, pH 7 to [M] 10 millimolar (mM) sodium-phosphate, pH 7 by adding 10 mL of [M] 1 Molarity (M) sodium-phosphate, pH 7 to an acid washed HDPE bottle containing 990 mL Milli-Q H_2O. Store at 4 °C.

4.6. Right before sampling, make a fresh sodium-phosphate protease inhibitor cocktail mixture in a metal free 15 mL tube.

4.7. Add 1 cOmplete™ ULTRA protease inhibitor cocktail tablet to 10 mL of [M] 10 millimolar (mM) sodium-phosphate, pH 7 . Keep the cocktail on wet ice.

1 Collect 10^8 - 10^9 cells by centrifugation (10000 x g, 4°C, 00:02:00) using Globe Scientific metal free 15 mL or 50 mL tubes. Discard the supernatant. 2m

2 Wash the cells by resuspending the cell pellet in 400 µL of trace metal grade [M] 10 millimolar (mM) sodium-phosphate, pH 7 and protease mixture. Collect cells by centrifugation (see step 1) and remove the supernatant with a P1000 pipette tip.

3 Resuspend the cells in 300 µL of trace metal grade [M] 10 millimolar (mM) sodium-phosphate, pH 7 and protease mixture. Transfer the cell suspension to cold 1.5 mL screw cap tubes containing acid washed beads.

3.1 The suspension will be thick and sticky. To collect the rest of the cell suspension, add an additional 100 µL of the sodium-phosphate and protease mixture to wash the sides of the tube and transfer all material to the respective 1.5 mL tube containing glass beads. Cells may stick to the P1000 tip so use a P100 to add the extra 100 µL of sodium-phosphate and protease mixture.

4 Optional: Flash freeze cell suspension in liquid nitrogen and store in -80 °C

4.1 Carefully fill a tube rack with liquid nitrogen to a level where half of the tube is submerged.

4.2 Make sure tubes are not tightly closed and air is allowed to pass through with the cap on.

4.3 Use tongs to place samples into the liquid nitrogen for 00:00:10 . 10s

4.4 Store samples in -80 °C until further processing.

Protocol references

Camacho, D. J., Perrino, C., and Merchant, S. S. (2024) HEPES-phosphate medium for growth of *Auxenochlorella protothecoides*, suitable for studies of trace element nutrition. Protocols.io

Quinn, J. M., & Merchant, S. (1998). [18] Copper-responsive gene expression during adaptation to copper deficiency. In *Methods in enzymology* (Vol. 297, pp. 263-279). Academic Press.