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

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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 H₂O.

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 $\times g$.
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 $\times g$.
3. 1 L HDPE bottle, 4 bottles.
4. Ultra-pure 6 M HCl.
5. Ultra-pure ICP-MS grade Milli-Q H₂O.
6. Trace metal grade Na₂HPO₄ anhydrous (dibasic, MW = 138 g/mol).
7. Trace metal grade NaH₂PO₄ • H₂O (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 μ m.
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.
3. 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  10 millimolar (mM) sodium-phosphate solution,  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  1 Mass Percent NaH_2PO_4 by adding  138 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ to a  1 L bottle with stirring and fill to  1 L with Milli-Q H_2O . Store at 4 °C.
 - 4.3. Make  1 Mass Percent 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  1 Mass Percent sodium-hosphate solution,  7 by mixing  390 mL of  1 Mass Percent NaH_2PO_4 and  610 mL of  1 Mass Percent Na_2HPO_4 . Store at 4 °C.
 - 4.5. Dilute  1 Mass Percent sodium-phosphate,  7 to  10 millimolar (mM) sodium-phosphate,  7 by adding  10 mL of  1 Mass Percent sodium-phosphate,  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  10 millimolar (mM) sodium-phosphate,  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 μ L of trace metal grade  10 millimolar (mM) sodium-phosphate,  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 μ L of trace metal grade  10 millimolar (mM) sodium-phosphate,  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 μ L 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 μ L 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.