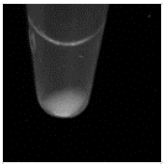

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Chlamydomonas reinhardtii cell wall proteins recrystallization

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocols describe the steps required for the recrystalization of cell wall proteins from *Chlamydomonas reinhardtii* from perchlorate extractions.

Protocol based on:

Goodenough, U. W. Gebhart, B. Mecham, R. Heuser, J. E. (1986). Crystals of the Chlamydomonas reinhardtii cell wall: Polymerization, depolymerization, and purification of glycoprotein monomers. Journal of Cell Biology. http://10.1083/jcb.103.2.405

PROTOCOL CITATION

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GUIDELINES

All steps described in this protocol are intended to be conducted in a research laboratory. Follow aseptic procedures.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Amicon Ultra-0.5 Centrifugal Filter Unit 30 KDa	UFC5030	Sigma Aldrich

SAFETY WARNINGS

Sodium perchlorate is a oxidizing agent, make sure to read the hazard information.

DISCLAIMER:

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Before start

1 Check the protocol "Chlamydomonas reinhardtii cell wall extraction with perchlorate"
The amount of recrystalized material varies, but in some results I was able to obtain 1% of the inicial cell mass as cell wall material.

Recrystalization can be performed by diafiltration of dialysis.

By Diafiltration 1h 30m

2 1h 30m

1. Prepare **1 mL** of cell wall proteins extract by perchlorate treatment of **1 x 10^9 cells** with **1 mL** of **2 Molarity (M)** sodium perchlorate
2. Add **500 µl** of sample in centrifugal filters with a 30 KDa cutoff (e.g. Amicon Ultra 0.5 Centrifugal filters)
3. Centrifuge at **14000 rcf, 25°C, 00:20:00**
4. Remove the flow-through and add enought sample to the max capacity of the filter. Repeat until all sample processed
5. Concentrate the sample until **100 µl**
6. Add 400 uL of ddH20
7. Centrifuge at **14000 rcf, 25°C, 00:20:00**
8. Repeat 4-7
9. Collect the precipitated material by inverting the membrane in a new collecting tube and centrifuge at **1000 rcf, 25°C, 00:01:00**
10. Proceed to planned experiments.

Amicon Ultra-0.5 Centrifugal Filter Unit 30 KDa
by Sigma Aldrich
Catalog #: UFC5030

By Dialsysis 2d

3 2d

1. Prepare membranes with 30 Kda for dialysis, by cutting a segment larger enough to hold **1 mL** of sample, and rehydrating it in ddH20 **00:30:00** prior to use
2. Prepare **1 mL** of cell wall proteins extract by perchlorate treatment of **1 x 10^9 cells** with **1 mL** of **2 Molarity (M)** sodium perchlorate,
3. Add the extract to the membrane adn close it
4. Place the membrane inside a vessel containig **1 L** of ddH20. *(A magnetic stirring can be used, making sure the membrane is not hit by the magnet bar)*
5. Incubate for **06:00:00**

6. Change the **1 L** of ddH20 and incubate **Overnight**
7. Retrieve the liquid inside the tube, ressuspending any precipitate material by pipetting and transfering it to a clean microcentrifugal tube.
8. Spin **20000 rcf, 25°C, 00:01:00** , a precipitate should be visible
9. Proceed to planned experiments.