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# *Chlamydomonas reinhardtii* cell wall proteins recrystallization

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## ABSTRACT

This protocols describe the steps required for the recrystallization 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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<https://protocols.io/view/chlamydomonas-reinhardtii-cell-wall-proteins-recry-bkrmkv46>

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## PROTOCOL INTEGER ID

41485

## 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.

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Before start

- 1 Check the protocol "[Chlamydomonas reinhardtii cell wall extraction with perchlorate](#)"  
The amount of recrystallized material varies, but in some results I was able to obtain 1% of the initial cell mass as cell wall material.

Recrystallization can be performed by diafiltration or dialysis.

By Diafiltration

1h 30m

- 2
  1. Prepare **1 mL** of cell wall proteins extract by perchlorate treatment of  **$1 \times 10^9$  cells** with **1 mL** of **2 Molarity (M)** sodium perchlorate <sup>1h 30m</sup>
  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 enough 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 ddH2O
  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 Dialysis

2d

- 3
  1. Prepare membranes with 30 Kda for dialysis, by cutting a segment larger enough to hold **1 mL** of sample, and rehydrating it in ddH2O **00:30:00** prior to use <sup>2d</sup>
  2. Prepare **1 mL** of cell wall proteins extract by perchlorate treatment of  **$1 \times 10^9$  cells** with **1 mL** of **2 Molarity (M)** sodium perchlorate,
  3. Add the extract to the membrane and close it
  4. Place the membrane inside a vessel containing **1 L** of ddH2O. (*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 ddH<sub>2</sub>O and incubate  **Overnight**
7. Retrieve the liquid inside the tube, resuspending any precipitate material by pipetting and transferring 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.