



Sep 02, 2020

Chlamydomonas reinhardtii cell wall extraction with perchlorate

Joao Vitor Molino¹

¹University of Zürich



This protocol is published without a DOI.



ABSTRACT

This protocols describe the steps required for the perchlorate extraction of cell wall proteins from Chlamydomonas reinhardtii

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

Joao Vitor Molino 2020. Chlamydomonas reinhardtii cell wall extraction with perchlorate. **protocols.io** https://protocols.io/view/chlamydomonas-reinhardtii-cell-wall-extraction-wit-bkpckviw

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Sep 02, 2020

LAST MODIFIED

Sep 02, 2020

PROTOCOL INTEGER ID

41412

GUIDELINES

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

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Sodium perchlorate	410241	Sigma Aldrich

SAFETY WARNINGS

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

DISCLAIMER:

DISCLAIMER - FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

BEFORE STARTING

Separate all material needed for the protocol. Read through the protocol.

Material

1 • [M] 2 Molarity (M) Sodium Perchlorate



Sodium perchlorate

by Sigma Aldrich

Catalog #: 410241 CAS Number: 7601-89-0

- Centrifuge tubes
- Pipettes and tips
- Centrifuge

Perchlorate solution

2 Sodium perchlorate has the following safety concerns.





Sodium Perchlorate

Prepare the solution in ddH20, by slowly adding the desired amount of Sodium perchlorate to ddH20

Cell preparation

30m

3 1. Grow cells until the beginning of the stationary phase. (*Until obtained the highest amount of cells*)

30m

- 2. Count the cells concentration, and harvest the target amount of cells by centrifugation

 (34000 rcf, 25°C, 00:10:00 . ([M]1 x 10^8 cells to [M]10 x 10^8 cells per mL of [M]2 Molarity (M)
 - ©4000 rcf, 25°C, 00:10:00 . ([M]1 x 10^8 cells to [M]10 x 10^8 cells per mL of [M]2 Molarity (M] sodium perchlorate were tested)
- 3. Remove the supernatant decantation or pipetting
- 4. Wash the cells with fresh TAP media, by ressuspending them in fresh media, followed by centrifugation

 34000 rcf, 25°C, 00:10:00
- 5. Remove the supernatant decantation or pipetting

Cell wall extraction

- 4 1. Add the desired amount of sodium perchlorate solution. (*ex. From a* [M]1 x 10^7 cells/mL *culture, harvest by centrifugation* 1.5 mL *of culture, and after washing, add 150 uL of sodium perchlorate solution*)
 - 2. Mix by vortexing or pipetting
 - 3. Centrifuge at max speed (ex. For smal volumes **320000 rcf, 25°C, 00:01:00** . For larger volumes lower speed can be used for longer time **4000 rcf, 25°C, 00:10:00**)
 - 4. Recover the supernatant containing the extracted proteins.