



Product Sheet

# *Chlamydomonas reinhardtii* (ATCC® 18302™)

Please read this **FIRST**



## Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

## Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Chlamydomonas reinhardtii* (ATCC® 18302™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
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Fax: 703.365.2750  
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## Description

**Deposited Name:** *Chlamydomonas reinhardtii* Dangeard

**Depositor:** M Nishimura

**Isolation:**

## Notes

This is a mutant strain which should be grown in the absence of light. Its growth rate is atypically slow for the genus, and it has been found to grow best on agar media, with cells closely grouped together. Also, this is a xenic culture (contains bacteria).

## Propagation

### Growth Conditions

**Temperature:** 25.0°C

Axenic

Prevent exposure to light during growth

### Medium

ATCC® Medium 277: Medium for chlamydomonas mutant

### Instructions for Complete Medium

ATCC Medium 277

## Protocols

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to the surface of a 20 x 100 mm Petri plate containing 20 ml of ATCC medium 277 agar. Wrap the plate culture with parafilm and incubate upright at 25°C in the dark. Alternatively, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing 5 ml of ATCC Medium 277 broth. Incubate the tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 25°C in the dark.

### Culture Maintenance

1. For a plate culture, transfer cells with an inoculating loop to a plate of fresh agar medium from a growing culture at or near peak density. For a broth culture, inoculate a tube of fresh broth medium with 0.1 ml from a growing culture at or near peak density.
2. Incubate at 25°C in the dark, with the cap loosened one half turn in the case of a test tube culture.
3. Subculture as necessary (i.e., every 4-6 months on agar media).

## Cryopreservation

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cells to  $2 \times 10^6$  -  $2 \times 10^7$ /ml in fresh medium.
3. While cells are centrifuging prepare a 10% (v/v) solution of sterile methanol in fresh medium.
4. Mix the cell preparation and the 10% methanol in equal portions. Thus, the final concentration will be  $10^6$  -  $10^7$  cells/ml and 5% (v/v) Methanol. The time from the mixing of the cell preparation and methanol stock solution to the beginning of the freezing process should be no less than 5 min and no greater than 15 min.
5. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this



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apparatus is approximately  
-1°C/min.)

7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. Vials should not be stored above -55°C.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to a centrifuge tube containing 5 ml of ATCC medium 277 broth (without agar). Centrifuge at 300 x g for 5 min.
10. Remove most of the supernatant (=methanol, which can inhibit growth) and then resuspend the pellet. Transfer the culture to the surface of an ATCC medium 277 agar plate (20 x 100 mm Petri plate containing 20 ml of ATCC medium 277 agar), or alternatively to a 16 x 125 mm screw-capped test tube containing 5 ml of ATCC medium 277 broth.
11. Incubate the culture at 25°C in the dark.

**References**

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

**ATCC Warranty**

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**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).  
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