



Product Sheet

# *Chlorella protothecoides* (ATCC® 30420™)

Please read this FIRST

Storage Temp.  
**Frozen Cultures:**  
-70°C for 1 week;  
liquid N<sub>2</sub> vapor  
for long term  
storage



**Freeze-dried Cultures:**  
2-8°C

**Live Cultures:**  
See Protocols  
section for  
handling  
information



Biosafety Level  
1

## 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: *Chlorella protothecoides* (ATCC® 30420™)

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Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
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Or contact your local distributor

## Description

**Strain Designation:** UTEX 130 [CCAP-211/6]  
**Deposited Name:** *Chlorella protothecoides* Kruger  
**Depositor:** UTEX  
**Isolation:** *Paramecium bursaria*, 1934

## Propagation

**Growth Conditions**  
**Temperature:** 25°C  
**Culture System:** Axenic

**Medium**  
ATCC® Medium 847: Algal proteose agar

## Protocols

### Storage and Culture Initiation

This strain is distributed as a freeze-dried preparation. If the culture will not be rehydrated immediately upon arrival, store at 5°C until processed.

1. To rehydrate an ampule, aseptically add 0.5 mL of ice-cold medium 847 broth containing 12% sucrose to the freeze-dried inner shell vial.
2. Once completely rehydrated, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing 5 mL of ATCC medium 847 broth. Incubate the tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 25°C under a 14 hour light (~50 μEinsteins/m<sup>2</sup>/s irradiance)/10 hour dark cycle. Alternatively, add the entire thawed contents to the surface of a 20 x 100 mm Petri plate containing 20 mL of ATCC medium 847 agar. Wrap the plate culture with parafilm and incubate upright under the same light/dark cycle as specified for a test tube culture.

**Note:** Some algae grow poorly or not at all when recovered from a dried state in broth media. In such cases, the algae should be recovered on agar media.

### 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 under a 14 hour light (~50 μEinsteins/m<sup>2</sup>/s irradiance)/10 hour dark cycle, with the cap loosened one half turn in the case of a test tube culture.
3. Subculture every 14-21 days.

## Cryopreservation

### Harvest and Preservation

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 x 10<sup>6</sup> - 2 x 10<sup>7</sup>/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<sup>6</sup> - 10<sup>7</sup> 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 cryovials (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 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




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- stored above -55°C.
- 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.
  - 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 847 without agar. Centrifuge at 300 x g for 5 min.
  - Remove most of the supernatant (=methanol, which can inhibit growth) and then resuspend the pellet. Transfer the culture to a 16 x 125 mm screw-capped test tube containing 5 mL of ATCC medium 847 broth or to the surface of an ATCC medium 847 agar plate (20 x 100 mm Petri plate containing 20 mL of ATCC medium 847 agar).
  - Incubate the culture at ~50 μEinsteins/m<sup>2</sup>/s irradiance at 25°C. Maintain under a 14/10h light-dark photoperiod.



**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**

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org)

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