



Product Sheet

# *Chlorella pyrenoidosa* (ATCC® 75668™)

Please read this FIRST



Storage Temp.  
**Frozen: -70°C or colder**  
**Freeze-Dried: 2°C to 8°C**  
**Live Culture: See Protocols Section**

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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 pyrenoidosa* (ATCC® 75668™)

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Manassas, VA 20108 USA  
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## Description

**Strain Designation:** UV232-1

**Depositor:** Bio-Technical Resources, JA Doncheck, Bio-Technical Resources

**Isolation:** Not applicable

## Propagation

### Growth Conditions

**Temperature:** 25°C

**Culture System:** Axenic

### Medium

ATCC® Medium 5: Sporulation agar

## Protocols

### Storage and Culture Initiation

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 sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing 5 mL of ATCC Medium 5 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  $\mu$ Einsteins/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 5 agar. Wrap the plate culture with parafilm and incubate upright under the same light/dark cycle as specified for a test tube culture. **Note:** Centrifugation immediately upon thawing (to remove most of the residual cryoprotectant) may aid in recovery of some strains (see steps 9-10 under the Cryopreservation section). Additionally, some algae grow poorly or not at all when recovered from the frozen state in broth media. In such cases, the algae should be recovered on agar media.

### Culture Maintenance

1. Screw the cap on tightly and vigorously agitate the culture.
2. Aseptically transfer a 0.1 mL aliquot to 5 mL of fresh medium in a 16 x 125 mm screw-capped test tube.
3. Screw caps on loosely (loosened one-half turn) and incubate on a 15° horizontal slant at 25°C under a 14 hour light (~50  $\mu$ Einsteins/m<sup>2</sup>/s irradiance)/10 hour dark cycle.
4. 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.



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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.

- 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 5 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 5 broth or to the surface of an ATCC medium 5 agar plate (20 x 100 mm Petri plate containing 20 mL of ATCC medium 5 agar).
- Incubate the culture at 50-100 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.

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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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