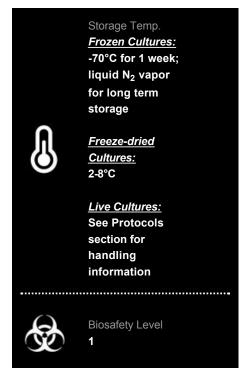


Product Sheet

Chlorella sp. (ATCC[®] 30562[™])

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: *Chlorella sp.* (ATCC[®] 30562[™])

American Type Culture Collection PO Box 1549 Manassas, VA 20108 USA www.atcc.org

800.638.6597 or 703.365.2700 Fax: 703.365.2750 Email: <u>Tech@atcc.org</u>

Or contact your local distributor

Description

Strain Designation: Syngen 2-3 Deposited Name: Chlorella sp.

Depositor: D Weis

Isolation: Paramecium bursaria 2/III, Cleveland, OH, 1976



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 it 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 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/m²/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.

Culture Maintenance

- 1. Screw the cap on tightly and vigorously agitate the culture.
- 2. Aseptically transfer a 0.1mL aliquot to 5 mL of fresh medium in a 16 x 125 mm screw-capped test tube.
- Screw caps on loosely (loosened one-half turn) and incubate on a 15° horizontal slant at 25°C under a 14 hour light (~50 μEinsteins/m²/s irradiance)/10 hour dark cycle.
- 4. Subculture every 14-21 days.



Cryopreservation

Harvest and Preservation

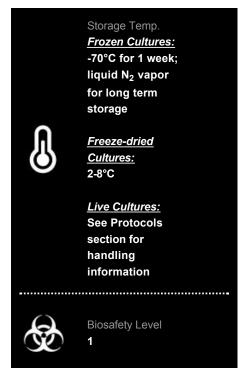
- 1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min.
- 2. Adjust the concentration of cells to 2×10^6 2×10^7 /mL in fresh medium.
- 3. While cells are centrifuging prepare a 10% (v/v) solution of sterile methanol in fresh medium.
- 4. Mix the cell 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 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 stabile indefinitely. Those stored at temperatures above



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- -130°C are progressively less stabile 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.
- Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the
 ampule and inoculate a 16 x 125 mm screw-capped test tube containing 5 mL of ATCC medium 847
 broth or to the surface of ATCC medium 847 agar plate (20 x 100 mm Petri plate containing 20 mL of
 ATCC medium 847 agar).
- 10. Incubate the culture on a 15° horizontal slant at 25°C with the cap screwed on loosely (loosened one-half turn) and incubate under a 14 hour light (~50 µEinsteins/m²/s irradiance)/10 hour dark cycle. Alternatively, add the entire contents of the thawed ampule to the surface of a 20 x 100 mm Petri plate containing 20 mL of ATCC medium 847 agar. Spread the material evenly over the plate with a sterile cooled spread bar. Incubate in the same manner as the test tube culture.



References

References and other information relating to this product are available online at 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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