



Product Sheet

Chlorella variegata (ATCC®) 30409™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ 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 variegata* (ATCC® 30409™)

American Type Culture Collection
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Or contact your local distributor

Description

Strain Designation: UTEX 28 [CCAP-211/10a]
Deposited Name: *Chlorella variegata* Beijerinck
Depositor: UTEX
Isolation: Freshwater, (?)

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. See the general procedures for opening a freeze-dried vial.

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/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. For a broth culture, screw the cap on tightly and vigorously agitate the culture. Aseptically transfer a 0.1 mL aliquot to 5 mL of fresh medium in a 16 x 125 mm screw-capped test tube. For a plate culture, transfer cells to a fresh plate with an inoculating loop.
2. Loosen the cap on a test tube culture and incubate on a 15° horizontal slant at 25°C under a 14 hour light (~50 μEinsteins/m²/s irradiance)/10 hour dark cycle. Wrap a plate culture with Parafilm and incubate upright under the same light/dark cycle as specified for 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 10⁶ - 2 x 10⁷/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 10⁶ - 10⁷ 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 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



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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 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 µEinstein/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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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

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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