



# ***Chlorella saccharophila* (Kruger) Migula var. *saccharophila***

**30408™**

## **Description**

**Strain designation:** UTEX 27 [CCAP-211/9a]

**Deposited As:** *Chlorella saccharophila* (Kruger) Migula var. *saccharophila*

**Type strain:** No

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## **Storage Conditions**

**Product format:** Freeze-dried

**Storage conditions:** 2°C to 8°C

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## **Intended Use**

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

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## **BSL 1**

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

***Chlorella saccharophila* (Kruger) Migula var.  
*saccharophila***  
**30408**

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

### Medium:

ATCC Medium 847: Algal proteose agar

**Temperature:** 25°C

**Culture system:** Axenic

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## Handling Procedures

### 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

## ***Chlorella saccharophila* (Kruger) Migula var. *saccharophila***

30408

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  $\mu\text{Einsteins}/\text{m}^2/\text{s}$  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 broth culture, inoculate a tube of fresh broth medium with 0.1 mL from a growing culture at or near peak density. 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.
2. Incubate at 25°C under a 14 hour light (~50  $\mu\text{Einsteins}/\text{m}^2/\text{s}$  irradiance)/10 hour dark cycle. In the case of a broth culture, screw tube cap on loosely (loosened one-half turn) and incubate on a 15° horizontal slant.
3. Subculture as necessary (i.e., every 14-21 days in broth 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

## ***Chlorella saccharophila* (Kruger) Migula var. *saccharophila***

30408

ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately  $-1^{\circ}\text{C}/\text{min.}$ )

7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below  $-130^{\circ}\text{C}$  are stable indefinitely. Those stored at temperatures above  $-130^{\circ}\text{C}$  are progressively less stable as the storage temperature is elevated. Vials should not be stored above  $-55^{\circ}\text{C}$ .
8. To establish a culture from the frozen state place an ampule in a water bath set at  $35^{\circ}\text{C}$ . 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 847 broth (without agar). Centrifuge at  $300 \times g$  for 5 min.
10. 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).
11. Incubate the culture at  $50\text{--}100 \mu\text{Einsteins}/\text{m}^2/\text{s}$  irradiance at  $25^{\circ}\text{C}$ . Maintain under a 14/10h light-dark photoperiod.

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### **Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Chlorella saccharophila* (Kruger) Migula var. *saccharophila* (ATCC 30408)

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### **References**

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

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### **Warranty**

## ***Chlorella saccharophila* (Kruger) Migula var. *saccharophila***

30408

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***Chlorella saccharophila* (Kruger) Migula var.  
*saccharophila***  
**30408**

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