



Product Sheet

# *Klebsormidium* *subtilissimum* (ATCC®) 30444™

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: *Klebsormidium subtilissimum* (ATCC® 30444™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

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## Description

**Strain Designation:** UTEX 176 [CCAP-335/3]  
**Deposited Name:** *Hormidium nitens* Meneghini  
**Depositor:** UTEX  
**Isolation:**  
freshwater, (?)

## Propagation

**Growth Conditions**  
**Temperature:** 25.0°C  
Duration: axenic

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

**Instructions for Complete Medium**  
ATCC Medium 847

## Protocols

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 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 mEinsteins/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.

## 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 mEinsteins/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

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 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.)



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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 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 without agar. Centrifuge at 300 x 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 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**

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**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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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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