



***Haematococcus lacustris* (Girod-Chantrons) Rostafinski**

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Description

Strain designation: UTEX 16 [CCAP-34/1b]

Deposited As: *Haematococcus lacustris* (Girod-Chantrons) Rostafinski

Type strain: No

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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.

BSL 1

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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.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:

ATCC Medium 847: Algal proteose agar

Temperature: 25°C

Culture system: Axenic

Handling Procedures

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**

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-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 (~50-100 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ 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.

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 a test tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 25°C under a 14 hour light (~50-100 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ 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.

Cryopreservation:

1. Harvest cells from a culture which is at or near peak density by adding 3.0 mL fresh ATCC medium 847 broth to the slant and washing cells into suspension.
2. Adjust the concentration of cells to $4 \times 10^6/\text{mL}$ with fresh broth medium, then dilute to half this concentration by adding an equal amount of a 10% (v/v) sterile methanol solution in fresh ATCC medium 847 broth.
3. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell preparation and the methanol solution, before the cooling cycle begins, should be no greater than 15 min.
4. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules 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.)

5. 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 can be stored between -80 and -70°C for no longer than one week.
6. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
7. 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.
8. Remove most of the supernatant (=methanol, which can inhibit growth) and then resuspend the pellet. Transfer the culture to a fresh tube of ATCC medium 847 and incubate on a 15° horizontal slant at 50-100 µEinstein/m²/s irradiance at 25°C with the cap loosened one half turn. Maintain under a 14/10 h light-dark photoperiod. 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Haematococcus lacustris* (Girod-Chantrons) Rostafinski (ATCC 30402)

References

References and other information relating to this material are available at www.atcc.org.

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Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor