ATCC

Product Sheet

Euglena gracilis var. bacillaris (ATCC® 50472™)

Please read this FIRST

Storage Temp.
Frozen: -70°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Protocols Section

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: *Euglena gracilis var. bacillaris* (ATCC® 50472™)

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
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Or contact your local distributor

Description

Strain Designation: W(3)BUL
Deposited Name: *Euglena gracilis* var. *bacillaris* Pringsheim
Depositor: JA Schiff
Isolation: Not applicable

Propagation

Growth Conditions
Temperature: 25°C
Culture System: Axenic

Medium
ATCC® Medium 351: Hutner's medium for Euglena
ATCC® Medium 1909: Hutner's Low pH Euglena Medium

Instructions for Complete Medium
Media: ATCC Medium 351. Addition of 0.1% sodium acetate to ATCC medium 351 may improve growth of some mutant strains of *Euglena* sp.

Alternate Media: ATCC Medium 1909

Protocols

Handling of Live Culture
This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 5 mL. When the culture arrives remove it promptly from the shipping container. Do not store the culture at refrigeration temperatures before handling. To assure viability, immediately loosen the test tube cap and incubate upright at 25°C for at least one hour before observing the culture. There should be numerous active trophozoites in suspension. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, aseptically transfer a 0.5 mL aliquot to a 16 x 125 mm screw-capped test tube containing 5 mL of sterile ATCC medium 351. Incubate the parent and daughter cultures upright with the caps on loosely at 25°C.

Culture Maintenance
1. Inoculate a tube of fresh broth medium with 0. 2 mL from a growing culture at or near peak density.
2. Incubate on a horizontal slant at 50-100 µEinsteins/m^2/s irradiance at 25°C with the cap loosened one half turn. Maintain under a 14/10 h light-dark photoperiod.

Cryopreservation

Harvest and Preservation
1. Harvest cells from a culture which is at or near peak density by centrifuging at 100 x g for 1 minute. Note: Centrifugation at the lowest speed and for the shortest time to allow sedimentation of the cells will maximize recovery.
2. Adjust the concentration of cells to 4 x 10^6/mL with fresh broth medium.
3. Transfer the concentrated cell suspension to a sterile Petri dish and allow the cells to remain undisturbed for at least one hour.
4. Transfer the cell suspension (note the volume) from the Petri plate to a 15 mL plastic centrifuge tube.
5. Add an equal volume of 6% (v/v) sterile methanol solution that has been prepared in fresh ATCC medium 351 broth. Mix gently but thoroughly.
6. 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 to the start of the cooling cycle should be no greater than 15 min.
7. 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.)
8. 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 can be stored between -80°C and -70°C for no longer than one week.

9. To establish a culture from the frozen state, aseptically add 0.5 mL fresh ATCC medium 351 broth to the frozen pellet, then place the ampule in a 35°C water bath until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
10. 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 351 broth. Incubate the tube upright for one hour at 25°C.
11. Gently remove as much supernatant as possible (the methanol cryoprotectant can inhibit growth) and refill with an equal volume of fresh broth medium.
12. Incubate on a horizontal slant at 50-100 µEinsteins/m^2/s irradiance at 25°C with the cap loosened one half turn. Maintain under a 14/10 h light-dark photoperiod. Note: Some strains may grow poorly or not at all when recovered from the frozen state directly into 5 mL of broth medium in a test tube. In such cases recovery may be improved by instead using a plate or flask containing a bed of ATCC medium 351 agar and gently increasing the volume of liquid medium incrementally by 1.0 mL every 10 min to a total of 8 mL. The plate or flask should be kept at a slight angle from the horizontal plane to pool the fluid to one side. Once motile cells are observed, they may be aseptically transferred to a single 16 x 125 mm screw-capped test tube containing 5 mL of ATCC medium 351 broth and incubated as indicated above.

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.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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

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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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