Euglena gracilis var. *bacillaris* Pringsheim

50470™

Description

Strain designation: M-(2)BUL
Deposited As: *Euglena gracilis* var. *bacillaris* Pringsheim
Type strain: No

Storage Conditions

Product format: Test tube
Storage conditions: See handling procedure

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

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.

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 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
Temperature: 25°C
Culture system: Axenic

Handling Procedures

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:

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 -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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Euglena gracilis* var. *bacillaris* Pringsheim (ATCC 50470)

References

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

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