Mastigamoeba aflagellifera

PRA-395™

Description

Strain designation: AF065-Y
Type strain: Yes

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

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 2832: Reduced YPD Medium
ATCC Medium 2154: LYI Entamoeba medium
Instructions for complete medium: Media: ATCC Medium 2832 may be prepared with an indicator that turns pink under low oxygen conditions. Use of rubber-seal screw caps on culture tubes helps minimize gas exchange, and filling tubes to the base of the tube neck with medium
Temperature: 10-30°C
Atmosphere: Microaerophilic
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 -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 sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a glass, rubber-seal screw-capped tube containing 14-15 mL ATCC Medium 2832. Screw cap on tightly and incubate on a 15° horizontal slant at 10-30°C (20-25°C recommended for routine cultivation).

Culture maintenance:

1. Ice culture at or near peak density for 10-15 min.
2. Vigorously invert culture 20-30 times or as necessary to sufficiently detach cells.
3. Aseptically transfer a 0.1 and 0.25 mL aliquot to fresh tubes of ATCC medium 2832.
4. Screw rubber-seal caps on tightly and incubate at a 15° horizontal slant at 10-30°C (20-25°C recommended for routine cultivation).
5. Subculture when many trophozoites are observed (typically every 2-5 days). The transfer interval will depend on the quantity of the inoculum, the incubation temperature, and the degree to which microaerophilic conditions are maintained inside the culture vessel. This interval should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized.

Reagents for cryopreservation: Reagents

Cryoprotective Solution

DMSO, 2.0 mL

Fresh growth medium, 8.0 mL

Cryopreservation:

1. Harvest cells from several cultures that are in the late logarithmic to early stationary phase of growth. Place culture vessels on ice for 20-30 min.
2. Vigorously invert tubes 20-30 times or as necessary to sufficiently detach cells, then centrifuge at 500 x g for 5 min. Handle cultures promptly after

centrifugation to avoid the amoebae reattaching to the culture tubes. **Note:** Increased yield may be obtained by using a sterile cotton swab to rub the inside surface of culture tubes both before and after centrifugation. Use of a refrigerated centrifuge will aid in preventing reattachment of cells to culture tubes during or immediately following centrifugation.

3. Adjust the concentration of cells to between 5 x 10^5/mL - 5 x 10^6/mL using reduced medium (i.e., supernatant).
4. Mix the cell preparation and the cryoprotective solution in equal portions. Invert the tube several times to obtain a uniform cell density.
5. Dispense 0.5 mL aliquots into 1.0 - 2.0 ml plastic sterile cryules (special plastic vials for cryopreservation).
6. Place the vials in a controlled rate freezing unit. Use the following cooling cycle: From room temperature cool at -10°C/min to the heat of fusion; from the heat of fusion to -40°C, cool at -1°C/min. At -40°C plunge into liquid nitrogen. The cooling cycle should be initiated no less than 15 and no more than 30 minutes after the addition of DMSO to the cell preparation.
7. Store ampules in a liquid nitrogen refrigerator until needed.
8. To establish a culture from the frozen state, place an ampule in a 35°C water bath, until thawed (2-3 min). Immerse the vial just sufficiently to cover the frozen material. Do not agitate the ampule.
9. Aseptically transfer contents of thawed ampule to a glass, rubber-seal screw-capped tube containing 14-15 mL ATCC Medium 2832.
10. Screw cap on tightly and incubate on a 15° horizontal slant at 10-30°C (20-25°C recommended for routine cultivation). Observe the culture daily and transfer when many trophozoites are observed.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Mastigamoeba aflagellifera* (ATCC PRA-395)

References

References and other information relating to this material are available at

www.atcc.org.

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Revision

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