ATCC

Product Sheet

Mastigamoeba aflagellifera (ATCC® PRA-395™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

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: *Mastigamoeba aflagellifera* (ATCC® PRA-395™)

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
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: AF065-Y
Depositor: A Tonouchi
Isolation: Soil of a rice field, Kanagi, Goshogawara city, Aomori pref., Japan, Dec. 1, 2006

Propagation

Growth Conditions
Temperature: 10°C to 30°C
Atmosphere: Microaerophilic

Medium
ATCC® Medium 2832: Reduced YPD Medium

Instructions for Complete Medium
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 produces microaerophilic conditions within 1-2d.

Protocols

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.

Cryopreservation

Reagents
Cryoprotective Solution
DMSO, 2.0 mL
Fresh growth medium, 8.0 mL

Harvest and Preservation

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.,

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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.

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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