Mastigella radicula (Moroff) Goldschmidt

50342™

Description

Strain designation: AJC/RS/35
Deposited As: *Mastigella radicula* (Moroff) Goldschmidt
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

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 802: Sonneborn's Paramecium medium
Instructions for complete medium: ATCC Medium 802 inoculated with *Enterobacter aerogenes* (ATCC® 13048™)
Temperature: 25°C
Atmosphere: Microaerophilic
Culture system: Xenic

Handling Procedures

Storage and Culture Initation
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. One day before thawing the ampule, prepare bacterized ATCC medium 802 i.e., inoculate medium with a bacteriological loop of *Enterobacter aerogenes* (ATCC® 13048™) from a nutrient agar slant (ATCC medium 3).
2. To thaw a frozen ampule, place it 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.
3. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 12 mL of bacterized ATCC Medium 802. Incubate the culture at 25°C.
   NOTE: Recovery of this culture from cryopreserved material may be particularly slow, in some cases requiring 2 weeks or more to reach peak culture density.

Culture maintenance:

1. Prepare bacterized ATCC medium 802.
2. When the culture is at or near peak density, rub the surface of the tube with a sterile cotton swab and vigorously shake the swab to dislodge the cells. Screw the cap on tightly and invert gently 10 times to distribute cells evenly.
3. Transfer approximately 0.25 mL to a T-25 tissue culture flask containing 10 mL of fresh ATCC medium 802.
4. Screw the caps on tightly and incubate at 25°C.

Cryopreservation: Harvest and Preservation

1. Harvest the cells from a culture that is at or near peak density by centrifuging at 850 x g for 5 minutes.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between 2 x 10^6 and 2 x 10^7cysts/mL with fresh medium. If the concentration is too low, centrifuge at 850 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 20% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.

*NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.

4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10^6 10^7 cells/mL and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge 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.)
7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.
9. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 12 mL of bacterized ATCC Medium 802. Incubate the culture at 25°C.

Notes

In addition to the food-source bacteria added to the growth medium, this xenic culture may contain the original bacterial flora present when the amoeba was first isolated.

Material Citation

If use of this material results in a scientific publication, please cite the material in the

following manner: *Mastigella radicula* (Moroff) Goldschmidt (ATCC 50342)

References

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

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