ATCC

Product Sheet

Acanthamoeba sp. (ATCC® PRA-228™)

Please read this FIRST

Biosafety Level
2

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: *Acanthamoeba sp.* (ATCC® PRA-228™)

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: 5a2
Depositor: S Schmitz-Esser
Isolation:
Lake sediment, Lake Neusiedl, Burgenland, Austria

Propagation

Growth Conditions
Temperature: 20.0°C
Growth condition: axenic
Protocol: This strain is shipped frozen. Thaw the frozen material in a 35C water bath and transfer the contents into a T25 flask containing 10ml of growth medium. Monitor for up to 3-4 weeks until the culture has reached peak density. To subculture, shake flask and transfer one tenth of the culture into a new flask with fresh medium.

Medium
ATCC® Medium 663: PYG medium

Instructions for Complete Medium
ATCC Medium 712

Culture Maintenance
1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.25 ml to a fresh tube or flask containing 5 ml of fresh ATCC medium 712.
3. Screw the caps on tightly and incubate at 30°[r2] C (incubate test tubes at a 15° horizontal slant).
4. The amoebae will form an almost continuous sheet of cells on the bottom surface of the flask or test tube.
Repeat steps 1-3 at 10-14 day intervals.

Cryopreservation

Storage temperature: liquid nitrogen vapor phase
1. To achieve the best results set up cultures with several different inocula (e.g. 0.25 ml, 0.5 ml, 1.0 ml). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density.
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 600 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 15% (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 and 10^7 cells/ml and 7.5% (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 remove the contents of the ampule and inoculate into 5 ml of fresh ATCC medium 712 in a T-25 tissue culture flask or plastic 16 x 125 mm screw-capped test tube. Incubate at 30°C.
Cryoprotective solution: 7.5% DMSO in growth medium mixed with Dryl's solution (ATCC medium 5080) at a

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1:1 ratio.

References

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

Biosafety Level: 2

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

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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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