



Product Sheet

Monosiga ovata (ATCC® 50635™)

Please read this **FIRST**



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: *Monosiga ovata* (ATCC® 50635™)

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
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Description

Strain Designation: M-1
Deposited Name: *Monosiga ovata* Kent
Depositor: AP Mylnikov, T Cavalier-Smith
Isolation:
freshwater pond, Yaroslavl, Russia, 1979

Propagation

Growth Conditions

Temperature: 25.0°C

Protocol: ATCCNO: 50633 SPEC: The strain is routinely distributed as a frozen stabulate. Thaw the ampule and aseptically transfer the material to a T-25 tissue culture flask containing 10 ml of ATCC medium 802. The bacterial flora that is present in the shipped culture will support growth. Growth may be improved by using bacterized ATCC medium 802 with a single species of bacteria. Bacterization is accomplished by inoculating the medium with *Enterobacter aerogenes* ATCC 13048 approximately 24 hours before use. Other species of bacteria may work equally well but this must be empirically assessed. The culture is routinely passaged every 14 days. Although this strain does form cysts they are not stable for long periods. To subculture, agitate and aseptically transfer 0.25 ml to 10 ml of freshly bacterized medium in a T-25 flask. Screw the cap on tightly and incubate at 25C.

Medium

ATCC® Medium 802: Sonneborn's Paramecium medium

Instructions for Complete Medium

ATCC Medium 802 inoculated with *Klebsiella pneumoniae* (ATCC® 700831).

Culture Maintenance

1. Prepare bacterized ATCC medium 802.
2. Inoculate a T-25 tissue culture flask containing 10.0 ml of bacterized ATCC medium 802 with 0.1 ml from a *Monosiga* culture at or near peak density.
3. Incubate at 25°C with cap screwed on tightly.
4. For routine maintenance subculture every 10-14 d.

Cryopreservation

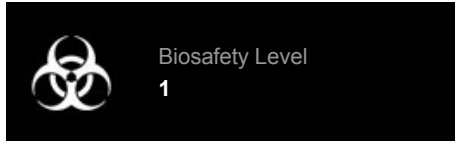
1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cells to $2 \times 10^6 - 10^7$ /ml in fresh medium.
3. While cells are centrifuging prepare a 20% (v/v) solution of sterile DMSO in fresh medium.
 - a) Add 2.0 ml of DMSO to an ice cold 20 x 150 mm screw-capped test tube;
 - b) Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 8.0 ml of ice cold medium;
 - c) Invert several times to dissolve the DMSO;
 - d) Allow to warm to room temperature.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be $10^6 - 10^7$ 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 should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. If frozen material is to be stored at temperatures between -130°C and -70°C the shelf life should be empirically tested, i.e., remove stored material at intervals to determine die-off rate.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the ampule to a level just above the surface of the frozen material. Do not agitate the ampule.



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9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a T-25 tissue culture flask containing 10 ml of bacterized ATCC medium 802.
10. Incubate with the cap screwed on tightly at 25°C.



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

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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