



Product Sheet

Monosiga brevicollis (ATCC® PRA-258™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ 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: *Monosiga brevicollis* (ATCC® PRA-258™)

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: MX1
Depositor: Nicole King
Isolation: ATCC® 50154™

Notes

This culture should only be fed a single species of feeder bacterium, *Flavobacterium* sp., which is included in the culture.

Propagation

Growth Conditions
Temperature: 25°C

Medium
ATCC® Medium 1525: Seawater 802 medium

Instructions for Complete Medium
ATCC Medium 1525 uninoculated with any feeder bacteria

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 it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. Add the thawed contents to a T-25 flask containing 10 mL of uninoculated ATCC medium 1525.
3. Incubate with the cap tightly sealed at 25°C.

Culture Maintenance

Subculture every two weeks to a fresh T-25 flask of uninoculated medium in the following manner:

1. Vigorously agitate the flask (or scrape the flask bottom using a sterile cell scraper) and aseptically transfer 0.5 mL from a growing culture to a T-25 tissue culture flask containing 10.0 mL of uninoculated ATCC medium 1525.
2. Incubate flask at 25°C with the cap on tightly.

Cryopreservation

Reagents

Cryoprotective Solution
DMSO: 2.0 mL
Fresh growth medium w/o bacteria: 8.0 mL

Harvesting and Preservation

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest cells from a culture that is at or near peak density by filtration and centrifugation at 800 x g for 5 min.
3. Adjust the concentration of cells at least 2 x 10⁶/mL in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
5. Dispense in 0.5 mL aliquots into 1.0 mL to 2.0 mL sterile plastic screw-capped cryovials (special plastic vials for cryopreservation).
6. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If



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- freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules 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.)
- Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
 - To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 mL uninoculated ATCC medium 1525.
 - Incubate at 25°C with the cap screwed on tightly.
 - Once the culture is established, vigorously agitate the flask and aseptically transfer 0.5 mL to 10.0 mL of uninoculated ATCC medium 1525.
 - Follow the protocol for maintenance of culture.



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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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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