ATCC

Product Sheet

Vermistella antarctica (ATCC® PRA-216™)

Please read this FIRST

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: *Vermistella antarctica* (ATCC® PRA-216™)

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: S-241
Depositor: DM Moran
Isolation:
Sediment at 290 meter depth near the Ross Ice Shelf, Antarctica

Propagation

Growth Conditions
Temperature: 4.0°C
Growth condition: Grown with Enterobacter aerogenes ATCC 13048 as food source

Medium
ATCC® Medium 1525: Seawater 802 medium
ATCC® Medium 1405: HESNW medium

Instructions for Complete Medium
ATCC® Medium 1525 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048); this media may be diluted with ATCC® Medium 1405 as needed, to keep the bacteria in check.

Protocols

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 ATCC medium 1525 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
3. Add one sterile wheat grain to the flask.
4. Incubate with the cap tightly sealed at 4°C.

Culture Maintenance
Subculture every two weeks to a fresh T-25 flask of bacterized medium in the following manner:
1. 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 ATCC medium 1525 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
2. Incubate with the cap tightly sealed at 4°C.
3. If the bacterial level of the culture is such that the organism is difficult to discern, it may be diluted to a ratio of
1:3 [1525 (bacterized):1405] as described in step 4.
4. Aspirate all media, as the amoebae will be adherent to the plastic (do not aspirate the wheat grain). For a T25 flask, add 6 mL ATCC Medium 1405 and 3 mL ATCC Medium 1525 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048). Incubate at 4°C with the cap tightly closed.

Cryopreservation

Cryoprotective Solution

DMSO	**2.0 ml**
Fresh growth medium w/o bacteria	**8.0 ml**

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.

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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 to at least 2 x 10^6/ml in fresh medium.
4. Mix the cell preparation and 20% (v/v) DMSO in equal portions. The final concentration will be 1.0 - 2.0 x 10^6 cells/ml and 10% DMSO. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 60 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 vials in a controlled rate freezing unit. From room temperature, cool at -1°C/min to -40°C. If 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.)
7. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. 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 ATCC medium 1525 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
9. Incubate at 4°C with the cap screwed on tightly.
10. Once the culture is established, vigorously agitate the flask and aseptically transfer 0.5 ml to 10.0 ml of bacterized ATCC medium 1525.
11. 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

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

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

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