



Product Sheet

Thraustochytriidae g. sp. (ATCC® PRA-301™)

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: *Thraustochytriidae g. sp.* (ATCC® PRA-301™)

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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Or contact your local distributor

Description

Strain Designation: 1616
Depositor: R Smolowitz
Isolation:
Hard clam (*Mercenaria mercenaria*)
Wellfleet Harbor, Cape Cod, MA

Propagation

Growth Conditions
Growth condition: axenic
Temperature: 25.0°C

Medium
ATCC® Medium 2715: QPX Medium

Instructions for Complete Medium

ATCC Medium 2715

Culture Maintenance

Quahog Parasite Unknown (QPX) thraustochytrids produce a thick, stringy mucoid substance in culture that can make passaging the culture difficult. Use of an open flame during handling can help disrupt the mucoid strands and make aseptic transfers more manageable.

1. When the culture is at or near peak density, vigorously agitate the culture and scrape the bottom of the flask with a sterile cell scraper.
2. Transfer approximately 0.5 to 1.0 ml to a new flask containing 10 ml of fresh ATCC medium 2715.
3. Screw the cap on tightly and incubate at 25°C
4. Repeat steps 1-3 at 2-3 week intervals.

Cryopreservation

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 2×10^6 and 2×10^7 cells/ml with fresh medium. If the concentration is too low, centrifuge at $600 \times g$ for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration. Note that it may be necessary to decant excess mucoid material rather than aspirate with a pipette.
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 and 10^7 cells/ml and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process 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 $-1^\circ\text{C}/\text{min}$ to -40°C . If the freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ 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 $-1^\circ\text{C}/\text{min}$.)
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 10 ml of fresh ATCC medium 2715 in a T-25 tissue culture flask. Incubate at 25°C .

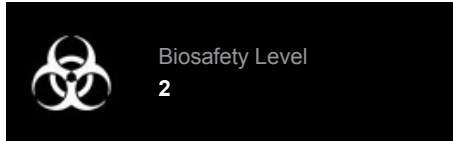
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References and other information relating to this product are available online at www.atcc.org.



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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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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