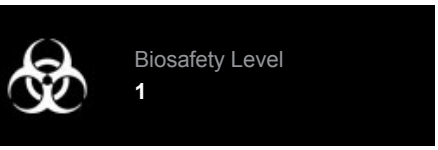
ATCC

Product Sheet

Didinium nasutum (ATCC® 30399™)

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: *Didinium nasutum* (ATCC® 30399™)

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

Deposited Name: *Didinium nasutum* (Muller) Stein
Depositor: W Balamuth
Isolation:
freshwater pond, San Francisco, CA, 1958

Propagation

Growth Conditions
Temperature: 25.0°C
Protocol: ATCCNO: 30399 SPEC: This strain is distributed as a dried preparation. See the general procedures for opening a dried vial. Aseptically, add 1 ml of ATCC medium 802 inoculated 24 hours previously with bacteria (e.g. Enterobacter aerogenes ATCC-13048) to the inner shell vial. Once completely rehydrated, aseptically transfer the material to a T-25 tissue culture flask containing a thriving culture of Paramecium, not provided. Place the flask at at 25C. Excystment should occur within a few days.

Medium
ATCC® Medium 802: Sonneborn's Paramecium medium

Instructions for Complete Medium
ATCC Medium 802 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).

Culture Maintenance
Subculture every 7-10 days to a fresh T-25 flask of bacterized medium in the following manner:
1. Aseptically transfer 1-2 ml from a growing culture to a T-25 tissue culture flask containing 10 ml of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
2. Aseptically transfer 1-2 ml from a thriving culture of *Paramecium* to the T-25 flask. Incubate the culture at 25°C with the cap screwed on tightly.

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.
2. Harvest *Didinium* cysts from a culture that has recently passed peak density by filtration and centrifugation at 800 x g for 5 min.
3. Adjust the concentration of cysts at least 2 x 10^4/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 - 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 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
9. Aseptically transfer 1-2 ml from a thriving culture of *Paramecium* to the T-25 flask. Incubate at 25°C with the cap screwed on tightly.
10. Once the culture is established, follow the protocol for maintenance of culture.

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Biosafety Level
1

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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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This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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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