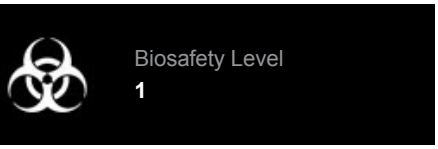
ATCC

Product Sheet

Nematocida sp. 1 (ATCC® PRA-372™)

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: *Nematocida sp. 1* (ATCC® PRA-372™)

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: ERTm6
Depositor: ER Troemel
Isolation:
Wild-caught Caenorhabditis briggsae, Cape Verde islands

Propagation

Growth Conditions
Culture System: *in-vivo* cultivation, *Caenorhabditis elegans* (Nematoda)

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 ampoules 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 1 minute. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. When completely thawed, dilute the spore preparation by addition of 0.25 to 0.5 mL of a balanced saline buffer solution such as ATCC medium 1323 Page's Balanced Saline, M9 buffer, or a Phosphate Buffered Saline (PBS; ATCC cat. 30-2200).
3. Infect *C. elegans* nematodes by adding the dilute spore preparation onto an agar plate containing an established *C. elegans* culture (stage L1 or L4/young adults). Seal the plate with parafilm and incubate upright at 25°C. The worms become infected by simply ingesting the spores. Follow the protocol for maintenance in-vivo.

Culture Maintenance
MAINTENANCE OF HOST C. ELEGANS CULTURE:
C. elegans nematodes may be maintained at 25°C on agar plates of ATCC medium 997, NG, or similar media containing a lawn of *E. coli* bacteria. If desired, a uracil-requiring strain of E. coli (such as strain OP50-1, available from the Caenorhabditis Genetics Center, University of Minnesota) may be used on a growth medium containing limited uracil, thereby precluding bacterial overgrowth which may obscure view of the nematodes.

NG agar

Agar (Difco 214010)	17.0 g
NaCl	3.0 g
Bacto peptone (Difco 211677)	2.5 g
Cholesterol (5 mg/mL)	1.0 mL
$CaCl_2$ (1 M)	1.0 mL
$MgSO_4$ (1 M)	1.0 mL
KH_2PO_4 (1 M)	25.0 mL

Mix the first three reagents in 800 mL of distilled water and autoclave. After the mixture is cool, add the last four reagents aseptically and adjust the volume of the medium to 1 L with sterile distilled water. See www.atcc.org for ATCC medium formulations. For further information on cultivation of *C. elegans* nematades, reference the following:
Brenner, S, 1974. Genetics 77: 71–94.
MAINTENANCE OF NEMATOCIDA CULTURE *IN-VIVO*:
Nematocida infection typically results in 50% host worms showing symptoms of infection at 2-3 d, and 50% host worm mortality at 4-5d. The infection may be propagated by transfer of a few (<10) infected host worms to a fresh plate culture of uninfected nematodes. Observe worms daily for symptoms of infection by preparing wet mounts for phase microscopy at approximately 600x (DIC imaging recommended). Nematocida infection manifests as distinct, granule-free regions within the *C. elegans* intestinal

Cryopreservation

M9 buffer

KH_2PO_4	3.0 g
Na_2HPO_4	6.0 g

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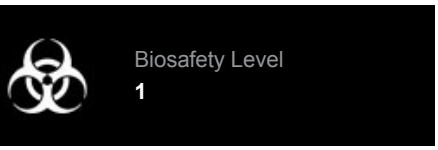

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NaCl	5.0 g
$MgSO_4$ (1M)	1.0 ml
Distilled H_2O	1.0 L

Autoclave 15 min. to sterilize.

See www.atcc.org for ATCC medium formulations.

An infectious extract of *Nematocida*-infected worms is prepared and cryopreserved as follows:

1. Harvest infected nematodes when most worms are filled with spores. Using a balanced saline buffer solution such as ATCC medium 1323, M9 buffer, or a PBS solution, wash nematode worms into suspension and transfer to a 15-ml centrifuge tube.
2. Centrifuge at 1500 x g for 30 sec, rinse 3 times with distilled water, let sit for 1 hr, then rinse again 2 times with distilled water.
3. Reduce volume of supernatant to ~1 ml, resuspend pelleted worms and transfer to a 2-ml microcentrifuge tube. Add ~500 µl silicon carbide beads (BioSpec Products, Inc. cat. 11079110sc) to the tube and vortex for 1 min, repeating four to five times.
4. Filter the worm lysate is through a 5 µm filter (Millipore) attached to a syringe in order to eliminate undisrupted *C. elegans* eggs, larvae, and other debris. (Filter becomes saturated after passing ~100 µl packed nematodes; use additional filters as necessary.)
5. Adjust the concentration of the filtrate containing *Nematocida* spores to 2.0 - 4.0 x 10^7 spores/ml with fresh buffer solution (i.e., ATCC medium 1323, M9 buffer, or a PBS solution).
6. Prepare a 30% (v/v) solution of sterile glycerol in fresh buffer solution.
7. Combine the filtrate and glycerol stock solutions in equal volumes to yield a final concentration of 1.0 - 2.0 x 10^7 spores/ml and 15% glycerol.
8. Dispense in 0.5 ml aliquots to 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
9. 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.)
10. Store in either the vapor or liquid phase of a nitrogen refrigerator.
11. 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 1 minute. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
12. When completely thawed, dilute the spore preparation by addition of 0.25 to 0.5 ml of a balanced saline buffer solution such as ATCC medium 1323, M9 buffer, or a PBS solution.
13. Infect *C. elegans* nematodes by adding the dilute spore preparation onto an agar plate containing an established *C. elegans* culture. Seal the plate with parafilm and incubate upright at 25°C. Follow the protocol for maintenance in-vivo.

For further information on cultivation/preservation of *Nematocida*, reference the following:

Estes, KA, et al., 2011. PLoS Pathogens 7(9): e1002227.

Troemel, ER, et al., 2008. PLoS Biology 6: 2736–2752.

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

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