



***Nematocida* sp. 1**

PRA-372™

Description

Strain designation: ERTm6

Type strain: No

Storage Conditions

Product format: Frozen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and

will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host:

in vivo cultivation, *Caenorhabditis elegans* (Nematoda)

Handling Procedures

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

CaCl₂ (1 M) 1.0 mL

MgSO₄ (1 M) 1.0 mL

KH₂PO₄ (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* nematodes, 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

Reagents for cryopreservation:M9 buffer

KH₂PO₄ 3.0 g

Na₂HPO₄ 6.0 g

NaCl 5.0 g

MgSO₄ (1M) 1.0 ml

Distilled H₂O 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. Cryopreservation:

2. 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.
3. 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.
4. 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.
5. 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.)
6. Adjust the concentration of the filtrate containing *Nematocida* spores to 2.0 - 4.0 x 10⁷ spores/ml with fresh buffer solution (i.e., ATCC medium 1323, M9 buffer, or a PBS solution).
7. Prepare a 30% (v/v) solution of sterile glycerol in fresh buffer solution.
8. Combine the filtrate and glycerol stock solutions in equal volumes to yield a final concentration of 1.0 - 2.0 x 10⁷ spores/ml and 15% glycerol.
9. Dispense in 0.5 ml aliquots to 1.0 - 2.0 ml sterile plastic screw-capped cryules

(special plastic vials for cryopreservation).

10. 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 $-1^{\circ}\text{C}/\text{min}$.)
11. Store in either the vapor or liquid phase of a nitrogen refrigerator.
12. 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.
13. 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.
14. 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Nematocida* sp. 1 (ATCC PRA-372)

References

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

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Revision

This information on this document was last updated on 2025-10-14

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