Nematocida parisii

PRA-289™

Description

Strain designation: ERTm1
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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*
Temperature: 25°C

Handling Procedures

Storage and Culture Initiation

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 may result in death of the culture.

1. Thaw a frozen ampule at room temperature. Immediately after thawing,

transfer contents to a lawn of *Escherichia coli* OP50-1 (Caenorhabditis Genetics Center, University of Minnesota) grown on a 6-cm NGM plate. Add synchronized *C. elegans* (L1s or L4/young adults) to the plate and incubate for 2 days at 25ºC.

2. Remove worms from the NGM plate and examine by DIC microscopy at 630X for the presence of meronts or spores.
   NOTE: A minimum of 20 worms should be examined for evidence of infection.

Culture maintenance:

1. Place 1-3 infected donor adult worms on a lawn of *Escherichia coli* OP50-1 grown on a 6-cm NGM plate.
2. Add 200–300 L1 recipient worms to the plate and co-incubate for 2 days at 25° C.
3. Remove worms and examine by DIC microscopy at 630X for the presence of meronts or spores.

Reagents for cryopreservation:

M9 buffer
KH_2PO_4, 3.0 g
Na_2HPO_4, 6.0 g
NaCl, 5.0 g
$MgSO_4$ (1M), 1.0 ml
Distilled H_2O, 1.0 L
Dissolve ingredients in 1 L of distilled water. Distribute 200 to 500 ml aliquots into appropriate sized bottles and autoclave for 15 minutes.

Cryopreservation:

1. To harvest the *Nematocida* culture, add 5 ml of M9 buffer to an infected NGM plate and transfer the suspension to a 15 ml centrifuge tube.
2. Centrifuge at 200 x g for 5 min. Remove the supernatant, resuspend the pellet in 5 ml of M9 buffer, and repeat the centrifugation step.
3. Repeat step 2 a minimum of five times in order to wash the infected worms.
4. After the last wash, resupend the pellet in 1 ml of M9 buffer and transfer the suspension to a 2 ml microcentrifuge tube. Add Silicon carbide beads (BioSpec Products, Inc.) to the tube and vortex for 1 minute. Repeat the procedure 4-5 times. Filter the worm extract through a Whatman filter paper number 1 to remove eggs and any remaining intact worms.
5. Perform a spore count of the worm extract and adjust the concentration to ≥ 3 x 10^7 spores/ml.
   NOTE: If the concentration of spores is too low, harvest infected worms from

additional NGM plates to yield the desired concentration.

6. Mix the extract with an equal volume of M9 buffer containing 30% glycerol. The final concentration of the extract will be ≥ 1.5 x 10^7 spores/ml and 15% glycerol.
7. Dispense 70 ml aliquots into 1.0-2.0 ml sterile plastic screw-capped cryovials.
8. 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.)
9. Store frozen ampules in either the vapor or liquid phase of a nitrogen refrigerator.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Nematocida parisii* (ATCC PRA-289)

References

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

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