Product Information Sheet for NR-22249

Anncaliia algerae, Undeen Isolate

Catalog No. NR-22249

For research use only. Not for human use.

NR-22249 is contaminated with *Mycoplasma* sp. Please determine whether or not this product is aceptable for your intended use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Microsporidia, Anncaliia*
Species: *Anncaliia algerae* (previously classified as *Nosema algerae* and *Brachiola algerae*)[1,2]
Strain: Undeen Isolate
Original Source: *Anncaliia algerae* (*A. algerae*), Undeen isolate was isolated from a mosquito, *Anopheles stephensi*, in 1970.[3]
Comment: The whole genome shotgun sequence of *A. algerae,* Undeen isolate, is available (GenBank: CAIR00000000).

Algerae is an obligate, amitochondriate intracellular parasite belonging to the order *Microsporidia. A. algerae* typically infects mosquitoes, but has also been identified as an opportunistic pathogen of humans in a similar fashion as other *Microsporidia.*[4] Many different isolates of *A. algerae* of insect and human origin have been established in culture since the 1920s.[5]

Material Provided:

Each vial of NR-22249 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-22249 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week. Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:

Eagle's Minimum Essential Medium (EMEM) supplemented with 10% heat inactivated fetal bovine serum (HIFBS) ATCC® medium 30-2003.
Rabbit kidney epithelial cells (ATCC® CCL-37™). Other cells lines that support growth of *A. algerae* include African green monkey kidney epithelial cells (ATCC® CCL-26™) and human lung fibroblasts (ATCC® CCL-75™).[5]

Incubation:
Temperature: 30°C
Atmosphere: 95% air, 5% CO_2

Propagation:

1. To establish a culture from the frozen state, place a vial in a 35°C to 37°C water bath. Thawing time is approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the contents to a tissue culture flask containing a fresh monolayer of rabbit kidney epithelial cells (ATCC® CCL-37™) and 10 mL of ATCC® medium 30-2003 containing 10% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
4. Incubate in a 30°C in a 95% air, 5% CO_2 incubator with the caps screwed on tightly. Observe the culture daily under an inverted microscope for the presence of spores growing inside parasitophorous vacuoles.

Maintenance:

1. Remove the medium from a fresh confluent monolayer of rabbit kidney epithelial cells in a tissue culture flask and replace it with 10 mL medium containing 10% (v/v) HIFBS.
2. Remove the medium from the *A. algerae* culture when approximately 50% of the rabbit kidney epithelial cell monolayer has lysed. Centrifuge the spores at 1300 x g for 10 minutes.
3. Remove the supernatant and resuspend the cell pellet in a small volume (0.5 to 1.0 mL) of ATCC® medium 30-2003 containing 10% (v/v) HIFBS or phosphate buffered saline (PBS). Transfer the resuspended pellet to the fresh flask of rabbit kidney epithelial cells, prepared in step 1 above. Follow steps 3 and 4 in Propagation.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Anncaliia algerae*, Undeen Isolate, NR-22249."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services,

Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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References:

1. Lowman, P. M., P. M. Takvorian and A. Cali. "The Effects of Elevated Temperatures and Various Time-Temperature Combinations on the Development of *Brachiola (Nosema) algerae* N. Comb. in Mammalian Cell Culture." J. Eukaryot. Microbiol. 47 (2000): 221-234. PubMed: 10847338.
2. Franzen, C., et al. "Transfer of the Members of the Genus *Brachiola* (Microsporidia) to the Genus *Anncaliia* Based on Ultrastructural and Molecular Data." J. Eukaryot. Microbiol. 53 (2006): 26-35. PubMed: 16441582.
3. Vavra, J. and A. H. Undeen. "*Nosema algerae* n. sp. (Cnidospora, Microspirida) a Pathogen in a Laboratory Colony of *Anopheles stephensi* Lison (Diptera, Culicudae)." J. Protozool. 17 (1970): 240-249. PubMed: 4915459.
4. Didier, E. S. and L. M. Weiss. "Microsporidiosis: Current Status." Curr. Opin. Infect. Dis. 19 (2006): 485-492. PubMed: 16940873.
5. Visvesvara, G. S. "*In vitro* Cultivation of Microsporidia of Clinical Importance." Clin. Microbiol. Rev. 15 (2002): 401-413. PubMed: 12097248.

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APPENDIX I: CRYOPRESERVATION

1. To harvest the *Anncaliia algerae* culture, detach any remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper.
2. Transfer the cell suspension (including parasites) to 15 mL plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.
3. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.
4. Pass the resulting cell suspension through a syringe equipped with a 27-gauge ½-inch needle to break up any remaining cells.
5. Adjust the parasite concentration to 2.0 to 4.0 x 10^7 cells/mL with fresh medium [Eagle's Minimum Essential Medium (EMEM) supplemented with 10% heat inactivated fetal bovine serum or Dulbecco's PBS (ATCC® 30-2200) can be used].
   Note: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 min and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
6. Mix equal volumes of parasite suspension and fresh medium or PBS containing 20% DMSO and 50% HIFBS to yield a final concentration of 1.0 to 2.0 x 10^7 cells/mL in 10% DMSO, 25% HIFBS. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the parasite suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 to 100 I.U./mL penicillin and 50 to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
8. Place the vials in a controlled rate freezing unit. From room temperature cool the vials 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 this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

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