Nosema trichoplusiae Tanabe and Tamashiro

30702™

Description

Deposited As: *Nosema trichoplusiae* Tanabe and Tamashiro
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

Instructions for complete medium:

Media: *in-vivo* cultivation, moth larvae

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 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 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. When completely thawed, infect lepidopteran larvae either by placing spores on the surface of their food plant or artificial diet, or by force-feeding with a syringe mounted on a micro-applicator. Follow the protocol for maintenance in vivo. The course of infection may be longer or shorter than usual depending on percent recovery of the parasite from the frozen state.

Culture maintenance:

When the frozen ampule arrives, store it as indicated above until ready to use. Each ampule contains 1.5 x 10^9 spores in a 0.5 ml 1:1 water-glycerol mixture. The number of spores should be sufficient to infect at least 10 larvae. For further information on growth and maintenance, refer to: J. Invertebr. Pathol. 9: 188-195, 1967; ibid., 24: 1-13, 1974.

Cryopreservation:

1. Harvest parasite spores according to the protocol for maintenance in vivo.
2. Centrifuge at 1400 x g for 10 min.
3. While spores are centrifuging prepare a 75% (v/v) solution of sterile glycerol in fresh Hank's Balanced Salt Solution (HBSS) (ATCC cat. 30-2101) or sterile distilled water.
4. Pool the pellets and adjust the concentration to 2.0 - 4.0 x 10^9 spores/ml with fresh HBSS or sterile distilled water.
   NOTE: If the concentration is too low centrifuge at 1400 x g for 10 min and resuspend in the volume of HBSS required to yield the desired concentration.
5. Mix the cell preparation and glycerol solution in a 1:3 ratio. The final concentration will be 1.0 - 2.0 x 10^9 spores/ml and 50% glycerol. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 30 min.
6. Dispense in 0.5 ml aliquots to 1.0-2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. 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.)

8. Store in either the vapor or liquid phase of a nitrogen refrigerator.
9. 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 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
10. When completely thawed, infect lepidopteran larvae either by placing spores on the surface of their food plant or artificial diet, or by force-feeding with a syringe mounted on a micro-applicator. Follow the protocol for maintenance in vivo.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Nosema trichoplusiae* Tanabe and Tamashiro (ATCC 30702)

References

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

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Revision

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