Besnoitia darlingi (Brumpt) Mandour

50978™

Description

Strain designation: OP1
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 2

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: CV-1 (ATCC CCL-70)
Medium:
ATCC Medium 2222: Cell Cultivation Medium for Parasites
Instructions for complete medium: Minimum essential medium (Eagle) (EMEM) (ATCC cat. 30-2003) supplemented with 10% fetal bovine serum (ATCC cat. 30-2020)
Temperature: 35°C

Handling Procedures

Cryopreservation:

1. Harvest the culture by gently agitating the contents of each flask. Transfer all but approximately 1 ml of the culture medium to 15 ml plastic centrifuge tubes. Detach the remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper. Pass the resulting cell suspension through a syringe equipped with a 27 gauge 1/2 in needle and pool this suspension with the

culture fluid.

2. Spin the cell suspensions at approximately 50 x g for 3 min, to remove the cellular debris.

3. Transfer the spore suspensions (supernatants) to new 15 ml plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.

4. Pool the spore pellets and adjust the concentration to 2.0 - 4.0 x 10^7 cells/ml with a fresh solution of Hank's Balanced Salt Solution.

*If the concentration is too low centrifuge at 1300 x g for 10 min and resuspend in the volume of Hank's Balanced Salt Solution required to yield the desired concentration.

5. Mix the spore preparation and 20% (v/v) DMSO in equal portions. The final concentration will be 1.0 - 2.0 x 10^7 cells/ml and 10% DMSO. 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. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC CCL-70 cells and 10 ml ATCC 30-2003 with 3% (v/v) HIFBS.

11. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.

12. Incubate in a 35°C CO_2 incubator with the caps screwed on tightly.

Notes

Culture is contaminated with *Mycoplasma* sp.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Besnoitia darlingi* (Brumpt) Mandour (ATCC 50978)

References

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

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