Crithidia mellificae Langridge and McGhee

30254™

Description

Crithidia mellificae is a parasitic protozoan that was isolated in 1974 from a honey bee in Athens, Georgia, United States.

Deposited As: *Crithidia mellificae* Langridge and McGhee

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

Medium:
ATCC Medium 355: Crithidia medium
ATCC Medium 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)
ATCC Medium 1373: TTYSH medium
Instructions for complete medium: Media: ATCC Medium 355.

Alternate Media: ATCC Medium 1034 can also be used for cultivation and is available in a freeze-dried format from ATCC (ATCC 327-X).
Temperature: 25°C
Culture system: Axenic

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 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
2. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 5 ml ATCC Medium 355.
3. Incubate upright at 25°C with caps screwed on tightly.

Culture maintenance:

1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.10 ml to a fresh tube containing 5 ml of fresh ATCC medium 355.
3. Incubate upright at 25°C with caps screwed on tightly.
4. Transfer every 14 days.

Cryopreservation:

1. Prepare a 10% (v/v) sterile DMSO solution in fresh ATCC Medium 355.
2. Transfer a culture at peak density to centrifuge tubes and centrifuge at 525 x g for 5 minutes.
3. Remove the supernatant and resuspend the cells in ATCC medium 355 to a concentration of 2 x 10^6 to 2 x 10^7 cells/ml.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10^6 and 10^7 cells/ml and 5% (v/v) DMSO.
5. Distribute the cell suspension in 0.5 ml aliquots into 1.0-2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.
6. 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.)

7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.
9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 5 ml of fresh ATCC medium 355 in a 16 x 125 mm screw-capped test tube. Incubate upright at 25°C with caps screwed on tightly.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Crithidia mellificae* Langridge and McGhee (ATCC 30254)

References

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

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