



Product Sheet

Crithidia mellificae (ATCC®) 30254™)

Please read this **FIRST**



Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Crithidia mellificae* (ATCC® 30254™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Deposited Name: *Crithidia mellificae* Langridge and McGhee

Depositor: RB McGhee

Isolation:

honey bee, *Apis mellifera*, Athens, GA, 1974

Propagation

Growth Conditions

Temperature: 25.0°C

Duration: axenic

Protocol: ATCCNO: 11745 SPEC: See general instructions for thawing and storage of frozen material before proceeding. Add thawed contents to a single 16 x 125 mm glass screw-capped test tube of the appropriate medium. Incubate the culture vertically with the cap screwed on tightly. It is essential to establish cultures initially in small volumes. Once established, the culture can be scaled up to larger volumes. Vigorously agitate the culture and aseptically transfer 0.1 ml of culture to a fresh tube of medium weekly.

Medium

ATCC® Medium 355: *Crithidia* medium

Instructions for Complete Medium

ATCC Medium 355

(ATCC medium 1034 can also be used for cultivation and is available in a freeze-dried format from ATCC; contact sales for details.)

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 2×10^6 to 2×10^7 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 $-1^\circ\text{C}/\text{min}$ to -40°C . If the freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ 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 $-1^\circ\text{C}/\text{min}$.)
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.

References

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

Biosafety Level: 1



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Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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