Strain Designation: Wallace
Deposited Name: Crithidia fasciculata Leger
Depositor: HN Guttman
Isolation: mosquito, Culex pipiens, St. Paul, MN, 1942

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 x 10⁶ to 2 x 10⁷ cells/ml.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10⁶ and 10⁷ 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.

References

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

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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