Deposited Name: *Dunaliella bardawil* Amotz and Avron
Depositor: A Ben-Amotz
Isolation: salt pond near Bardawil Lagoon, North Sinai, Israel, 1976

Growth Conditions
Max Temperature: 25.0°C
Min Temperature: 20.0°C

Instructions for Complete Medium
ATCC Medium 1174: DA medium

Culture Maintenance
1. Inoculate a test tube containing 5 ml of fresh medium with 0.1 ml from a growing culture at or near peak density.
2. Incubate at 50-100 µEinsteins/m²/s irradiance at 25°C. Maintain under a 14/10 h light-dark photoperiod.

Cryopreservation
1. Prepare a 5% (v/v) sterile methanol solution in fresh ATCC medium 1174 without agar.
2. Harvest cells from a culture which is at or near peak density by adding 3.0 ml methanol solution to the slant and washing cells into suspension.
3. Adjust the concentration of cells to 2 x 10⁶/ml with fresh methanol solution.
4. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell preparation and the methanol solution, before the cooling cycle begins, should be no greater than 15 min.
6. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen. 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. Store ampules in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. Shelf life must be determined empirically for storage temperatures above -130°C.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to a centrifuge tube containing 5 ml of ATCC medium 1174 without agar. Centrifuge at 300 x g for 5 min.
10. Remove most of the supernatant and then resuspend the pellet. Place culture on ATCC medium 1174 slants and incubate on a 15° horizontal slant at 50-100 µEinsteins/m²/s irradiance at 25°C. Maintain under a 14/10 h light-dark photoperiod.

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

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