Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level
1

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

Complete Growth Medium
Grace’s insect medium, 79%; heat-inactivated fetal bovine serum, 10%; whole egg ultrafiltrate, 10%; bovine plasma albumin (fraction V), 1%

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Antheraea cells (ATCC® CCL-80™)

Unpacking & Storage Instructions
1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells
To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor, not at –70°C. Storage at –70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 24°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete growth medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 24°C in a suitable incubator. CO₂ in air atmosphere is detrimental to cells when using the medium described on this product sheet.

Handling Procedure for Flask Cultures
The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination
2. Incubate the flask in an upright position for several hours at 24°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.
3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2 to 3 x 10⁶ viable cells/mL in the shipping medium.
4. Incubate the culture, horizontally, at 24°C in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

Product Sheet

Antheraea cells (ATCC® CCL-80™)

Please read this FIRST

**Storage Temp.**
- liquid nitrogen
- vapor phase

**Biosafety Level**
- 1

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**Complete Growth Medium**
Grace's insect medium, 79% heat-inactivated fetal bovine serum, 10%; whole egg ultrafiltrate, 10%; bovine plasma albumin (fraction V), 1%

**Citation of Strain**
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Antheraea cells (ATCC® CCL-80™).

The cells grow in suspension and subcultures are prepared by diluting 0.5 mL of cell suspension with 4.5 mL of fresh culture medium and dispensing into a new flask. Culture at 23°C to 25°C.

**Subcultivation Ratio:** A subcultivation ratio of 1:10 to 1:12 is recommended

**Medium Renewal:** At the time of subcultivation (approximately every 6 days)

**Cryopreservation Medium**
Complete growth medium described above supplemented with 10% (v/v) glycerol.

**Comments**

This cell line derived from ovarian tissues of the moth, Antheraea eucalypti, by Grace in 1962, constituted the first true line of arthropod cells established in cell culture. Because of the difficulty and expense in obtaining significant volumes of lepidopteran hemolymph, the Antheraea cells were adapted to hemolymph-free culture medium by Yunker, Vaughn and Cory. The cell line was grown in Grace’s insect tissue culture medium supplemented with 10% FBS (heat-inactivated), 10% whole chicken egg ultrafiltrate and 1% bovine plasma albumin (fraction V). The cells grow predominantly in suspension although some cells adhere to the vessel walls. Yunker and Cory found the adapted Antheraea cells were able to support the growth of a number of arboviruses.

**References**
References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

**Biosafety Level: 1**
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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