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 phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°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 culture medium and spin at approximately 125 x g for 5 to 10 minutes.
4. Resuspend cell pellet with the recommended complete 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 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if 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 37°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. Incubate the culture, horizontally, at 37°C in a 5% CO₂ in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.
**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**

Dulbecco’s modified Eagle’s medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose and supplemented with 5 mM HEPES, 95%; fetal bovine serum, 5%

**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: AMJ2-C11 (ATCC® CRL-2456™)

**Subculturing Procedure**

Firmly tap the flask against palm of hand to dislodge any attached cells and transfer along with the floating cells into new flasks.

**Medium Renewal:** Twice per week

**Cryopreservation Medium**

Complete growth medium described above supplemented with extra 5% FBS and 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Comments**

AMJ2-C8 (ATCC CRL-2455) and AMJ2-C11 (ATCC CRL-2456) are cloned, continuous, alveolar macrophage (AM) cell lines generated from C57BL6J mice by in vitro infection with the J2 retrovirus carrying the v-raf and v-myc oncogenes.

Flow cytometry detected the product of the raf gene in the cytoplasm of these cell lines. Studies on the tumoricidal properties of these cell lines demonstrated differences in their response to a panel of known macrophage activators.

AMJ2-C8 was activated following exposure to recombinant murine interferon gamma (rMuIFN-gamma) but not lipopolysaccharide (LPS) or muramyl dipeptide (MDP).

AMJ2-C11 most closely resembled the response pattern of the parental AM, since it could be activated by either the combination of rMuIFN-gamma plus LPS or rMuIFN-gamma plus MDP.

The cells retain many characteristics of alveolar macrophages. They are phagocytic, non-specific esterase positive and they express macrophage Mac-1 antigens and Fc receptors.

Constitutive expression of MHC-class-II antigens was low on AMJ2-C11 but was increased following exposure to rMuIFN-gamma.

The cell line did not secrete substantial amounts of IL-1 or TNF but did secrete large amounts of IL-6.

The cells produce nitric oxide (NO) when stimulated with a mixture of rMuIFN-gamma and LPS.

**References**

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

**Biosafety Level: 2**

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**

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**Disclaimers**

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

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