**Description**

Organism: *Mus musculus*, mouse  
Strain: BALB/c  
Cell Type: Monocyte, Macrophage  
Gender: male  
Growth Properties: adherent

**Batch-Specific Information**

Refer to the Certificate of Analysis for batch-specific test results.

**SAFETY PRECAUTION**

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

**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 7 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. Also, check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. If the cells are still attached, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.
RAW 264.7 gamma NO(-)
(ATCC® CRL-2278™)

**Please read this FIRST**

Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level 2

**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**

The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum (ATCC 30-2020) to a final concentration of 10%.

**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: RAW 264.7 gamma NO(-) (ATCC® CRL-2278™)

**Subculturing Procedure**

Cells can be grown as a monolayer or in spinner cultures. Routine passage cells should be grown on bacterial grade culture dishes. Subcultures are prepared by scraping cells from floor of dishes every two days and diluting to 1 x 10^6 cells/20 mL (3 x 10^5 cells/20 mL for weekend passage). Cells grown in spinner flasks are inoculated at a density of 1 x 10^6 cells/mL. Spinner culture cell densities should not be allowed to rise above 6 x 10^5 cells/mL.

**Medium Renewal:** 2 to 3 times per week

**Cryopreservation Medium**

Complete culture medium described above supplemented with 5% (v/v) DMSO.

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

**Comments**

Unlike the parental line, RAW 264.7 gamma NO(-) does not produce nitric oxide upon treatment with interferon gamma alone, but requires LPS for full activation (the iNOS promoter linked to a luciferase reporter gene is also unresponsive to IFN- alone). This property makes its behavior more like that of normal macrophages from some commonly used strains of mice.

**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**

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

**Disclaimers**

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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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