



I-13.35

CRL-2471™

Description

Organism: *Mus musculus*, mouse

Cell Type: macrophage

Tissue: Spleen

Age: adult

Gender: Female

Morphology: macrophage

Growth properties: Adherent

Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C

Handling Procedures

Unpacking and 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.

Complete 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, 70%; fetal bovine serum, 10%; LADMAC Conditioned Media (produced from the LADMAC cell line

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(CRL-2420), 20%

Handling Procedure:

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. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete medium and dispense into a 25 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.

Subculturing procedure:

1. Remove and discard 75% of culture medium.
2. Scrape cells with cell scraper.
3. Add appropriate aliquots of cell suspension to new culture vessels.
4. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended

Medium Renewal: Every 2 to 3 days

LADMAC Conditioned Medium

LADMAC conditioned medium is made from **LADMAC** cells (ATCC CRL-2420). LADMAC

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cells secrete the growth factor colony stimulating factor 1 (CSF-1). CSF-1 is capable of supporting the *in vitro* proliferation of mouse bone marrow macrophages. The Pannell-Milstein roller bottle apparatus may be used to produce high concentrations of CSF-1 [Ref](#)Olivas E, et al. Use of the Pannell-Milstein roller bottle apparatus to produce high concentrations of the CSF-1, the mouse macrophage growth factor. J. Immunol. Methods 182: 73-79, 1995.

Subculturing Procedure for LADMAC cells

Cultures can be established by centrifugation with subsequent resuspension at 2×10^5 viable cells/mL. Attached cells may be dispersed by tapping the sides of the flask until cells are loose.

Complete Growth Medium for LADMAC cells

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO₂ in air atmosphere.

Conditioned medium harvesting:

1. Allow LADMAC cells to become confluent.
2. After 5 to 7 days, collect supernate, centrifuge at 125 x g for 5 to 10 minutes
3. Filter (200 nM filter) and store aliquots at -20°C

Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: I-13.35 (ATCC CRL-2471)

References

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

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