ATCC

Product Sheet

M3/84.6.34 (ATCC® TIB-168™)

Please read this FIRST

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

RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, 80%; heat-inactivated fetal bovine serum, 20%
RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, 80%; heat-inactivated fetal bovine serum, 20%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: M3/84.6.34 (ATCC® TIB-168™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Rattus norvegicus* (B cell); *Mus musculus* (myeloma), rat (B cell); mouse (myeloma)
Isotype: rat IgG1 kappa
Cell Type: hybridoma: B lymphocyte
Morphology: lymphoblast
Growth Properties: suspension

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

HANDLING PROCEDURE FOR FROZEN CELLS

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 37°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point on should be carried out under strict aseptic conditions.

- The cells are supplied in two different types of glass ampules. One is a standard ampule, the neck of which must be scored with a sharp file that has been immersed in ethanol. A definitive sharp nick about 1/8" in length on one side is necessary. The second type is prescored and is identifiable by a gold band around the ampule neck, and should not be scored with a file.

- Break the neck of the ampule between several folds of a sterile towel.

- Transfer the cell suspension and dilute it with the recommended culture medium in a culture flask (see specific batch information above for dilution ratio); incubate at 37°C with 5% CO_2 in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the ampule contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO_2 will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

FLUID RENEWAL
Add fresh medium (depending on cell density) every 2-3 days.

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SUBCULTURE PROCEDURE
Cultures can be maintained by the addition of fresh medium or replacement of medium. Adherent cells can be dislodged by scraping and cultures established by centrifugation with subsequent resuspension at 1-2 x 10(5) viable cells/ml. Maintain culture density between 10(5) and 10(6) viable cells/ml.

Handling Procedure for Flask Cultures

HANDLING PROCEDURE FOR FLASK CULTURES (SUSPENSION)
The flask was seeded with cells (see specific batch information above for concentration), grown and completely filled with medium to prevent loss of cells in transit. Upon receipt incubate the flask in an upright position for several hours to return the flask contents to 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Resuspend the cell pellet in 10-12 ml of the shipping medium. From this suspension remove a sample for a cell count and viability so that the cell density of the suspension can be adjusted to 1-2 x 10(5) viable cells/ml. If the suspension needs to be diluted use the shipping medium. Incubate the culture in a flat position at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure described above.

Subculturing Procedure

Protocol: Cultures can be maintained by the addition of fresh medium or replacement of medium. Adherent cells can be dislodged by scraping and cultures established by centrifugation with subsequent resuspension at 1 to 2 X 10(5) viable cells/ml.
Interval: Maintain cell density between 1 X 10(5) and 1 X 10(6) viable cells/ml.
Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)

Comments

Animals were immunized with detergent solubilized mouse(C57BL/6) macrophage membrane which had been depleted of previously identified antigens with monoclonal immunoadsorbants.
Like Mac-2, the Mac-3 antigen is not expressed on bone marrow cells (Mac-1 is expressed on bone marrow cells).
Also like Mac-2, Mac-3 appears to be expressed on their monocytic line of differentiation at a stage after divergence from the granulocytic series.
Mac-2 and Mac-3 are present on 69% of macrophages and 0% to 2% of thymocytes.
Expression of Mac-3 is increased during the differentiation from monocyte to activated peritoneal macrophage.
Tested and found negative for ectromelia virus (mousepox).

References

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

ATCC Warranty

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

Disclaimers

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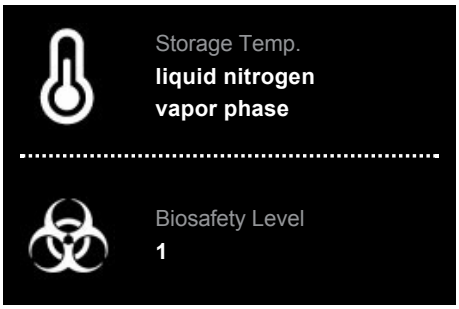

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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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