ATCC

Product Sheet

M1/70.15.11.5.HL (ATCC® TIB-128™)

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

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum 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: M1/70.15.11.5.HL (ATCC® TIB-128™)

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 IgG2b
Tissue: spleen
Cell Type: hybridoma: B lymphocyte
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 37øC 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 37øC with 10% CO2 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 CO2 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.

SUBCULTURE PROCEDURE
Cultures can be maintained by the addition of fresh medium or the 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 cell culture density between 10(5) and 10(6) viable cells/ml.

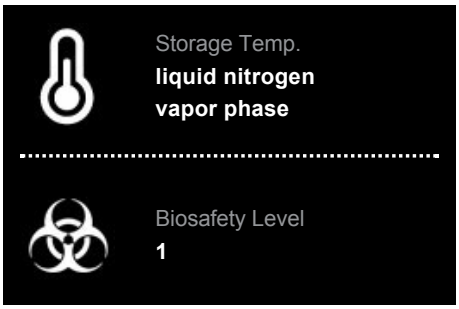
HANDLING PROCEDURE FOR FLASK CULTURES (SUSPENSION)
The flask was seeded with cells (see specific batch information above for

ATCC

Product Sheet

M1/70.15.11.5.HL (ATCC® TIB-128™)

Please read this FIRST



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 Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum 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: M1/70.15.11.5.HL (ATCC® TIB-128™)

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

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 37øC. 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 37øC. The shipping medium contains reduced sodium bicarbonate suitable for a 5% CO2 in air incubator. DMEM usually contains 3.7 grams of sodium bicarbonate per liter and should be incubated in a 10% CO2 in air incubator. Maintain the cell density of the culture as suggested under the subculture procedure described above.

NOTE
This material is available under the conditions that you will not use it for commercial purposes or distribute it to third parties.

CATALOGUE DESCRIPTION
This hybridoma secretes a monoclonal IgG2b antibody which reacts with the alpha chain of the murine macrophage-granulocyte specific antigen Mac-1 (CD116). Mac-1 is a macrophage differentiation antigen associated with type three complement receptor. The antigen defined by this hybridoma is expressed in large quantities on thioglycollate peritoneal exudate macrophages and in lesser amounts on neutrophilic granulocytes, blood monocytes, 8% of spleen cells, 44% of bone marrow cells, and less than 0.3% of thymus cells. This antibody precipitates 2 polypeptides of 190,000 and 105,000 daltons. This antibody labels human monocytes and polymorphonuclear leukocytes and a small population of lymphocytes. It is capable of both natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC). The hybridoma was formed by the fusion of mouse myeloma line NS-1 with spleen cells from DA rats immunized with C57BL/10 mouse spleen cells enriched for T lymphocytes. References: Eur. J. Immunol. 8: 539-551, 1978; ibid., 9: 301-306, 1979; J. Biol. Chem. 256: 3833-3839, 1981; Monoclonal Antibodies, R. Kennett, et al. (eds.), pp. 185-217, Plenum Press, 1980; J. Exp. Med. 158: 586, 1983. Originator: T. Springer, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA.

(9/95)

(Last page for TIB-128)

Subculturing Procedure

Protocol: Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be 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 C57BL/10 mouse spleen cells enriched for T lymphocytes.
Spleen cells were fused with NS-1 myeloma cells.
Mac-1 is a mouse macrophage differentiation associated with type three complement receptor (CR3).
The antigen is expressed in large amounts on thioglycollate induced peritoneal exudate macrophages and in lesser quantities on neutrophilic granulocytes, blood monocytes.
8% of spleen cells, 44% of bone marrow cells and less than 0.3% of thymus cells react with the antibody.
The antibody precipitates two polypeptides of 190000 and 105000 daltons, binds to human monocytes, polymorphonuclear leukocytes and a small population of lymphocytes.
The antibody is capable of both natural killing and antibody dependent cellular cytotoxicity.
Tested and found negative for ectromelia virus (mousepox).
The line was recloned by TA Springer in 1988, and a new seed stock was created at the ATCC.

References

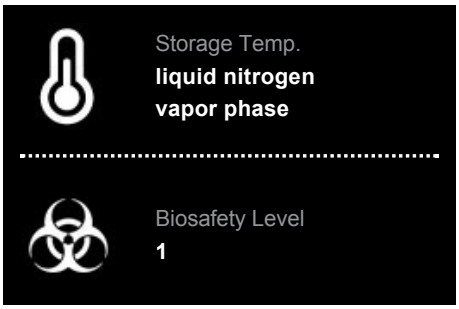
References and other information relating to this product are available online at www.atcc.org.

ATCC

Product Sheet

M1/70.15.11.5.HL (ATCC® TIB-128™)

Please read this FIRST



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 Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum 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: M1/70.15.11.5.HL (ATCC® TIB-128™)

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

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.
Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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

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