



M1/70.15.11.5.HL

TIB-128™

Description

Organism: *Rattus norvegicus* (B cell); *Mus musculus* (myeloma), rat (B cell); mouse (myeloma)

Cell Type: hybridoma: b lymphocyte

Tissue: Spleen

Growth properties: Suspension

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: 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%.

Handling Procedure:

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 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 $1-2 \times 10^5$ 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 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 $1-2 \times 10^5$ viable cells/ml. If the suspension needs to be diluted use the shipping medium. Incubate the culture in a flat position at 37°C. 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.

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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 2×10^5 viable cells/ml.

Interval: Maintain cell density between 1×10^5 and 1×10^6 viable cells/ml.

Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)

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: M1/70.15.11.5.HL (ATCC TIB-128)

References

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

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