ATCC

Product Sheet

3A5 (ATCC® CRL-2440™)

Please read this FIRST

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

HL-1 medium supplemented with 4 mM L-glutamine, 1 mM sodium pyruvate and 1% fetal bovine serum. HL-1 medium can be obtained from from Lonza (catalog number 77201).

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: 3A5 (ATCC® CRL-2440™)

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: *Mus musculus* (B cell); *Mus musculus* (myeloma), mouse (B cell); mouse (myeloma)
Isotype: IgM; kappa light chain
Cell Type: hybridoma:
Morphology: lymphoblast
Growth Properties: suspension

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

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.

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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 75 cm^2 tissue culture flask and dilute with the recommended complete culture medium (see the specific batch information for the recommended dilution ratio). 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 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).
4. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

It is not necessary to remove the cryoprotective agent. If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio recommended in the specific batch information.

Handling Procedure for Flask Cultures

Handling Procedure For Flask Cultures (Suspension)

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
2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 ml of this medium.
3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 1-2 x 10^5 viable cells/ml in the shipping medium.
4. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

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Subculturing Procedure

Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)
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 5 X 10 exp4 viable cells/ml.
Maintain cell density between 5 X 10 exp4 and 1 X 10 exp6 viable cells/ml.
Attached cells may be subcultured by tapping the sides of the flask until cells are dispersed.

Cryopreservation Medium

Cryoprotectant Medium

HL-1 Complete Serum-free medium described above supplemented with 20% (v/v) fetal bovine serum and 5% (v/v) DMSO.

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

Comments

The cell line was established by Jose A. Lebron and Pamela J. Bjorkman in 1997.
Animals were tolerized with human beta2-microglobulin and immunized with purified, soluble, human HFE.
Spleen cells were fused with HL-1 Friendly myeloma cells (a derivative of P3X63Ag8.653 that is deficient in both hypoxanthine phosphoribosyl transferase [HPRT] and adenine phosphoribosyl transferase [APRT]).
The antibody recognizes the HFE portion of the HFE/beta2 heterodimer.
HFE is a major histocompatibility complex (MHC) related protein that is mutated in the iron-overload disease hereditary hemochromatosis.
This antibody can be used for ELISA applications and to immunoprecipitate soluble HFE; it is not reactive in Western Blot assays.

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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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.
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