ATCC

Product Sheet

MB19tsA, clone 2B2 [Mbeta19tsA, clone 2B2] (ATCC® CRL-2308™)

Please read this FIRST

Biosafety Level
2

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

Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with 0.08 mg/ml hygromycin B, 90%; fetal bovine serum, 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: MB19tsA, clone 2B2 [Mbeta19tsA, clone 2B2] (ATCC® CRL-2308™)

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*, mouse
Cell Type: fibroblastSV40 large T antigen transfected
Age: 10.5 days gestation
Morphology: fibroblast
Growth Properties: adherent

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

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 **34°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 **34°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 (Monolayer)

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. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

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2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at **34°C** in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at **34°C** in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Subcultivation Ratio: A subcultivation ratio of 1:10 to 1:15 is recommended
Medium Renewal: Every 2 to 3 days
Remove medium, rinse quickly with 0.25% trypsin, 0.03% EDTA solution, add 1-2 ml additional trypsin solution and allow flasks to set at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new flasks.

Cryopreservation Medium

Cryoprotectant Medium
Complete culture medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

The genotype of this cell line has recently been determined to be DNA polymerase beta mutant (null) and DNA polymerase iota mutant (null).
MB19tsA, clone 2B2 is a fibroblast-like cell line derived from primary cultures of DNA polymerase beta (pol-beta) deficient fibroblasts isolated from embryos from heterozygous females (beta-polymerase +/-) at day 10.5 of gestation.
The pol-beta deficient fibroblasts were immortalized by transfection with the DNA plasmid construct ptsA58H which contains coding sequences for both a hygromycin-resistance gene and tsA58H, a temperature-sensitive SV40 T antigen.
Cells were selected in the presence of 0.080 mg/ml hygromycin for 21 days and further selected by limiting dilution.
The cell line is deficient in base excision repair due to homozygous deletion of the promoter and exon I in the pol-bet a gene.
The deletion of pol-beta is mediated by the Cre-loxP recombination.
This deletion event is reversible and therefore this cell line is ideal for site-specific recombination using the Cre-lox system.
The contributor of this line has demonstrated that insertion of a single-copy of a variety of cDNAs can be site-specifically reinserted at the pol-beta locus, and that this is a stable event.

References

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

Biosafety Level: 2

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