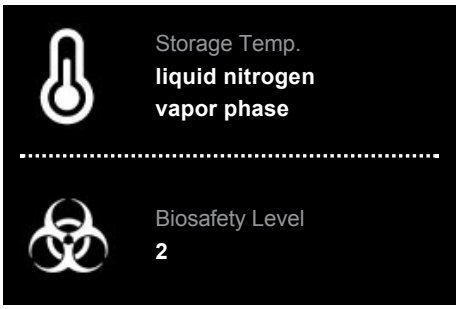
ATCC

Product Sheet

TF-1.CN5a.1 (ATCC® CRL-2512™)

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

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 supplemented with 2 ng/ml GM-CSF and 0.4 mg/ml G-418, 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: TF-1.CN5a.1 (ATCC® CRL-2512™)

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: *Homo sapiens*, human
Disease: erythroleukemia
Cell Type: erythroblast
Age: 35 years
Gender: male
Morphology: lymphoblast
Growth Properties: suspension
DNA Profile:
Amelogenin: X,Y
CSF1PO: 13
D13S317: 8,9
D16S539: 9,12
D5S818: 13
D7S820: 12
THO1: 7,9
TPOX: 8
vWA: 15,17

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 **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. It is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a 15 ml centrifuge tube and dilute with the recommended complete culture medium Centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium
4. Transfer the cells to an appropriate size vessel. 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).
5. 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

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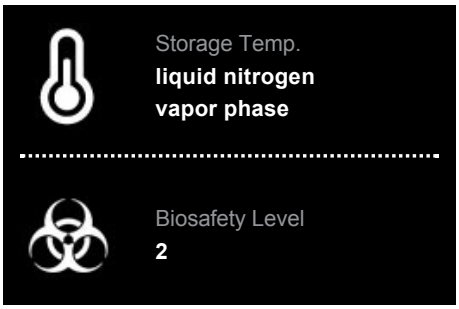

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Handling Procedure for Flask Cultures

Handling Procedure for Flask Cultures

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 2-3 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.

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 X 10(5) viable cells/ml.

Medium Renewal: Every 2 to 3 days

Cryopreservation Medium

Cryoprotectant Medium

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

Comments

The cells stably express the alpha subunit of human ciliary neurotrophic factor (CNTF) receptor and CNTF supports the short term proliferation of the cells. Suggested assay conditions are washing the cells with RPMI to remove GM-CSF, then incubation in RPMI 1640 95%, fetal bovine serum 5%, and picogram/ml concentrations of CNTF. The cells maintain responsiveness for at least 193 population doublings in full maintenance medium.

This cell line was derived from the TF-1 cell line (ATCC CRL-2003).

TF-1 cells were transfected, using Transfectam, with the gene for the alpha subunit of human ciliary neurotrophic factor (CNTF) receptor ligated to the pCR3.1 vector.

The vector contains cytomegalovirus (CMV) and SV40 viral sequences and the neomycin resistance gene.

Following transfection, a line of G418 resistant cells was obtained and named TF-1.CN5a.

TF-1.CN5a cells were grown, transiently, in the absence of GM-CSF and with 2 ng/ml CNTF to establish the TF-1.CN5a.1 cell line.

The cells can be used to access the potency and to study signal transduction of CNTF.

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

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

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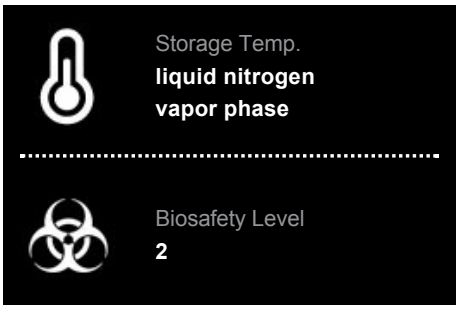

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longer valid.

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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