ATCC

Product Sheet

NIT-2 (ATCC® CRL-2364™)

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

Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 90%; heat-inactivated dialyzed 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: NIT-2 (ATCC® CRL-2364™)

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*, transgenic for SV40 large T antigen, mouse, transgenic for SV40 large T antigen
Disease: adenoma; carboxypeptidase E defective
Cell Type: beta cell
Age: 10 week old
Gender: male
Morphology: epithelial
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 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. 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).
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 37°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 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: Twice per week
Subcultures are prepared using a cell dissociation buffer (an enzyme free Hanks' based solution; Catalog number: 13150-016 available from GIBCO).
Remove the medium from the culture flask, add 2 ml of cell dissociation buffer per 25 sq. cm flask (5 ml per 75 sq. cm. flask and gently rock the flask at room temperature for 1 to 2 minutes to bathe the cells in the buffer. Aspirate the solution and discard. Allow the flask to sit at room temperature for 3 to 4 additional minutes (total time from initial addition of cell dissociation buffer is approximately 5 minutes).
Firmly tap the flask against the palm of the hand to dislodge cells.
Add 5 ml of fresh medium per 25 sq. cm. flask (10 ml per 75 sq. cm. flask) and triturate up and down directing the stream along the bottom of the flask to dislodge the cells and break up some of the clumps.
Add fresh medium, aspirate and dispense into new flasks.

Cryopreservation Medium

Cryoprotectant Medium

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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 NIT-2 cell line was derived from the pancreatic beta cells of Cpe(fat)/Cpe(fat) mice by crossing C57BLKS/J-Cpe(fat)/+ mice with NOD/Lt-Tg(RIPTag)1Lt mice.
NOD/Lt-Tg(RIPTag)1Lt mice are transgenic for the SV40 large T antigen under the control of a rat insulin promoter, and spontaneously develop beta adenomas.
Carboxypeptidase E is required for complete conversion of proinsulin to mature insulin. A spontaneous point mutation in the coding region of the carboxypeptidase E (CPE) gene in Cpe(fat)/Cpe(fat) mice affects proinsulin processing.
The NIT-2 cell line was cultured from adenomatous islets obtained from a 10 week old F2 male and was compared with the NIT-1 cell line (ATCC-CRL-2055) previously developed from mice with wild-type CPE.
Electron microscopy of the cultured NIT-2 showed increased numbers of enlarged and electron-lucent granules compared with NIT-1 cells.
Pro-CPE, but not the mature form of CPE, is present in NIT-2 cells, and neither pro-CPE nor CPE are secreted into the medium.
Proinsulin is less extensively processed in NIT-2 than in NIT-1 cells, indicating that the Cpe(fat)mutation affects both the endopeptidase and carboxypeptidase reactions.
The secretion of insulin/proinsulin from NIT-2 cells is significantly elevated by secretagogues, indicating that CPE is not required for sorting proinsulin into the regulated pathway.

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 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.
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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.