ATCC

Product Sheet

NIT-1 (ATCC® CRL-2055™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

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-1 (ATCC® CRL-2055™)

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
Strain: NOD/Lt
Tissue: pancreas/islet of Langerhans
Disease: insulinoma
Cell Type: beta cell
Age: 10 weeks
Gender: female
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 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.

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 centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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 complete 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.

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 x *g* 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

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are ready to be subcultured.

Subculturing Procedure

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 cm^2 flask (5 mL per 75 cm^2 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 cm^2 flask (10 mL per 75 cm^2 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.
Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: 1 to 2 times per week

Cryopreservation 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

These mice are transgenic for the SV40 large T antigen under the control of a rat insulin promoter, and spontaneously develop beta adenomas.
At passage 18, most cells stained positively for insulin, less than 5% were positive for glucagon and none were positive for somatostatin or pancreatic polypeptide.
Insulin secretion is responsive to glucose concentration in the medium.
There is low constitutive expression of MHC class I, class II and ICAM-1 mRNA, but expression of both is markedly increased by treatment with interferon gamma.
Stimulation of class I mRNA is accompanied by increased class I antigen expression and induction of an occult class I product expressing the H-2.39 specificity.
MHC class II antigen is not induced despite the induction of the mRNA.
NIT-1 cells show ultrastructural features of differentiated mouse beta cells (well developed rough endoplasmic reticulum, extensive golgi apparatus and beta granules).
The cells shed a mature ecotropic type C retrovirus. The retrovirus is capable of infecting other Fv-1 n mouse cell lines, so care should be taken to avoid cross infection.
NOTE: NIT-1 cells will not form a confluent monolayer; however, they will form nice colonies of monolayered cells in a fairly dense array.
When the NIT-1 colonies begin to ball up slightly and show many round cells on top of the monolayers as well as floating in the media, it is time to passage them.

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

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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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