NIT-2

CRL-2364™

Description

Organism: *Mus musculus*, transgenic for SV40 large T antigen, mouse, transgenic for SV40 large T antigen
Cell Type: beta cell
Tissue: Pancreas
Age: 10 weeks
Gender: Male
Morphology: epithelial
Growth properties: Adherent
Disease: Adenoma; Carboxypeptidase E defective

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies

and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Cells contain polyomaviral DNA sequences

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Unpacking and 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.

Complete 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%.

Handling Procedure: 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 growth medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete growth 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.

Subculturing procedure: Volumes used in this protocol are for 25 cm^2 or 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

NOTE: NIT-2 cells will not form a confluent monolayer, however, they will form nice colonies of monolayered cells in a fairly dense array. When the NIT-2 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.

1. Remove and discard culture medium.

2. Subcultures are prepared using a cell dissociation buffer (an enzyme-free Hanks' based solution; GIBCO, Catalog #13150-016). Add 2 mL cell dissociation buffer per 25 cm. sq. flask (5 mL per 75 cm. sq. flask) and gently rock flask to bathe the cells at room temperature for 1 to 2 minutes.
3. Aspirate the solution and discard.
4. Allow the flask to remain at room temperature for 5 additional minutes (total time from initial addition of cell dissociation buffer approximately 7 minutes).
5. FirmLy tap the flask against palm of hand to dislodge cells.
6. 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.
7. Add appropriate aliquots of cell suspension to new culture vessels.
8. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: 1:2 to 1:3
Medium Renewal: Twice weekly

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: NIT-2 (ATCC CRL-2364)

References

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

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