





Product Sheet

alphaTC1 Clone 9 (ATCC® CRL-2350™)

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

The base medium for this cell line is Dulbecco's Modified Eagle's Medium (Invitrogen Cat. No. 31600-034). To make the complete growth medium, add the following components to the base medium:

- heat-inactivated fetal bovine serum (FBS) to a final concentration of 10%
- 15 mM HEPES
- 0.1 mM non-essential amino acids
- 0.02% bovine serum albumin
- 1.5 g/L sodium bicarbonate
- 2.0 g/L glucose

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: alphaTC1 Clone 9 (ATCC® CRL-2350™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
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Description

Organism: *Mus musculus*, mouse

Strain: (C57BL/6J x DBA/2)F2

Tissue: pancreas

Disease: adenoma

Cell Type: alpha cell

Morphology: epithelial

Growth Properties: adherent, single cells and loosely attached clusters

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. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium. to one T-25. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ 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% CO₂ 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 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.


Subculturing Procedure




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Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

NOTE: Warm all solutions to 37°C prior to use.

1. Transfer all medium and floating cells from flask to a 50 mL centrifuge tube.
2. Adherent cells are removed using Cell Dissociation Buffer (an enzyme free buffer; Invitrogen, Catalog No. 13150-016). Add 5 mL of diluted cell dissociation buffer per 75 cm² flask and gently rock flask to bathe the cells at room temperature for 1 to 2 minutes.
3. Allow the flask to remain at room temperature for 1 to 5 additional minutes until cells have detached from the flask.
4. Firmly tap the flask against palm of hand to dislodge cells.
5. Add 10 mL of fresh medium per 75 cm² flask and triturate up and down directing the stream along the growth surface of the flask to dislodge the cells and break up some of the clumps.
6. Transfer these cells to the centrifuge tube from Step 1. Centrifuge at 125 x g for 5 to 10 minutes. Remove medium and resuspend pellet in fresh complete medium.
7. Add appropriate aliquots of cell suspension to new culture vessels.
8. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended.

Medium Renewal: 2 to 3 times a week.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.



Cryopreservation 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

Though the parental alphaTC1 cell line produces glucagon and considerable quantities of insulin and preproinsulin mRNA, the clonal line is more differentiated and produces glucagon but not insulin or preproinsulin mRNA.

Rat recombinant gamma-interferon or mouse recombinant interleukin-1 individually inhibited glucagon synthesis in alphaTC1 clone 9 cells but did not cause inhibition of DNA synthesis.

The combination of these cytokines caused marked inhibition of DNA and glucagon synthesis in alphaTC1 clone 9 cells.



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

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

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