Adherent

aseptically remove the entire contents of the flask and centrifuge at

If the cells are not attached,

aseptically remove all but 5 to 10 mL of the shipping medium. The

Biosafety Level

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature

Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep

Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at

If the cells are still attached,

Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by

Incubate the culture at 33°C in a suitable incubator. A 5% CO₂

Resuspend cell pellet with the recommended complete medium (see the specific batch information for

Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.

Check all containers for leakage or breakage.

It is important to note that some vials leak when submersed in liquid nitrogen

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

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

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

Handling Procedure for Flask Cultures

The flask was seeded with cells, incubated, and completely filled with medium at ATCC to prevent loss of

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.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be

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.

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phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.
Subculturing Procedure

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

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1X PBS (Ca²⁺/Mg²⁺-free, ATCC SCRR-2201) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of 0.25% (w/v) Trypsin / 0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 33°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of cell suspension to new culture vessels.

**Subcultivation ratio:** 1:4 – 1:8

6. Place culture vessels in incubators at 33°C.

**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.

**Medium Renewal**

Two to three times weekly

**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.

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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 ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 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: AFT024 (ATCC® SCRC-1007™)