



# hTERT EP156T

CRL-3289™

## Description

**Organism:** *Homo sapiens*, human

**Cell Type:** epithelial cell

**Tissue:** Prostate

**Gender:** Male

**Morphology:** epithelial-like

**Growth properties:** Adherent

**Disease:** Normal

**Cells per vial:** Approximately  $1.0 \times 10^6$

**Volume:** 1.0 mL

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## 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.

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## 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

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

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 submerged 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 submerged in liquid nitrogen.

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### Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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### Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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### 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

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ready for use.

**Complete medium:** MCDB-153 modified medium (Biological Industries, 01-059-1A) supplemented with 25 mg bovine pituitary extract (Gibco, 13028-014 **-or-** Sigma P1476-2.5ML), 1% Fetal Bovine Serum (FBS; ATCC 30-2020), 5 ng/ml huEGF (Gibco, PHG0311), and 500 ng/ml Puromycin (Gibco, A1138-03)

## Handling Procedure:

To ensure 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 8.0 mL complete culture medium.
4. Rinse the ampoule with 1.0 mL of complete culture medium and transfer the contents to the same centrifuge tube.
5. Pellet the cells by centrifugation at approximately 220 x *g* for 5 to 7 minutes, then remove the cryoprotectant.
6. Resuspend cell pellet with 1.0 to 2.0 mL complete medium and count cells. 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). pH (7.0 to 7.6).
7. Seed cells at an appropriate density and incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

## Subculturing procedure:

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks

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(catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer twice with D-PBS (ATCC 30-2200) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA for Primary Cells Solution (ATCC PCS-999-003) to flask, ensure complete coverage of cells and remove trypsin, then incubate and observe cells under an inverted microscope until cells have detached (usually within 2 to 5 minutes).
4. Once detached, add 2.0 to 3.0 mL of a 0.05% soybean trypsin inhibitor (ATCC 30-2104) and aspirate cells by gently pipetting. Transfer cell suspension into a 15ml conical tube.
5. Add 4.0 to 7.0 mL of complete growth medium to flask and aspirate cells by gently pipetting. Transfer suspension into previous 15ml conical tube.
6. Collect cells by centrifugation at 220xg for 5min.
7. Resuspend cell pellet in 1.5 to 3.0 mL of complete medium. Add appropriate aliquots of the cell suspension to new culture vessels.  
Cultures can be established between  $5 \times 10^3$  and  $1 \times 10^4$  viable cells/cm<sup>2</sup>.
8. Incubate cultures at 37°C.

**Interval:** Maintain cultures at a cell concentration between  $5 \times 10^3$  and  $5.0 \times 10^4$  cell/cm<sup>2</sup>.

**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:10 is recommended

**Medium Renewal:** 2 to 3 times per week

**Reagents for cryopreservation:** Complete growth medium supplemented with 6.5% (v/v) FBS and 10% (v/v) DMSO (ATCC 4-X)

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: hTERT EP156T (ATCC CRL-3289)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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