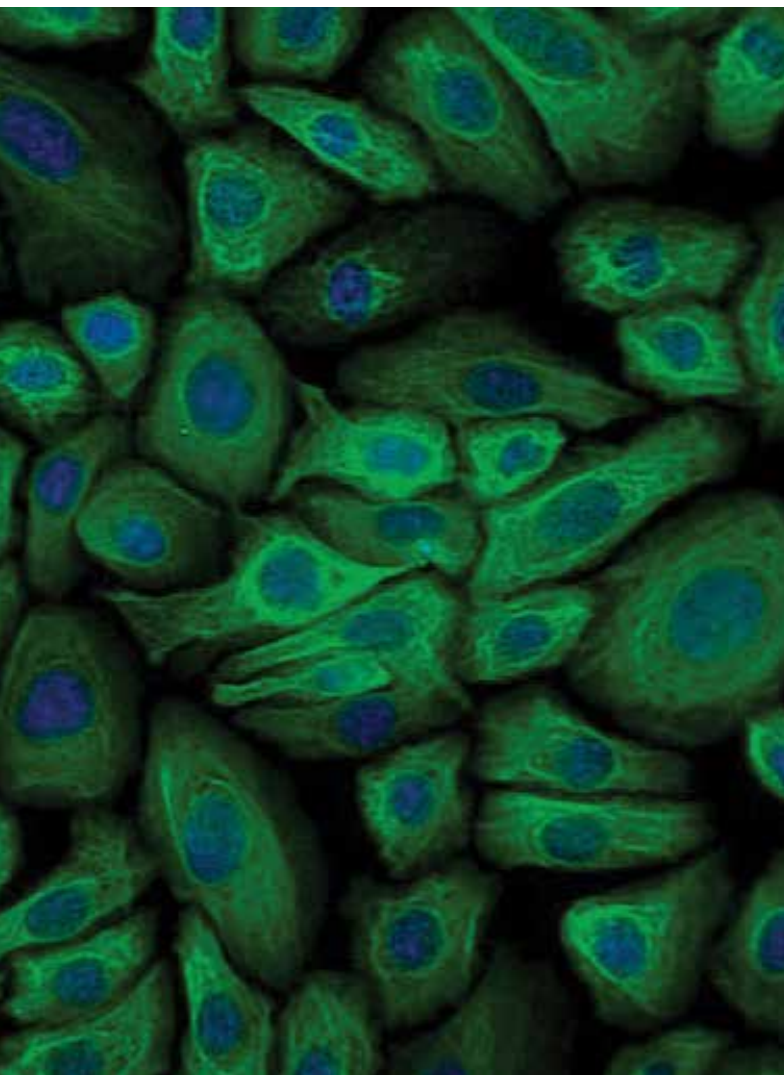


# ATCC® hTERT IMMORTALIZED CELL CULTURE GUIDE

tips and techniques for culturing hTERT immortalized cells



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# Telomeres and Telomerase

Telomeres are repetitive DNA sequences that stabilize the terminal ends of the chromosomes. During each cell division, 50-200 base pairs of DNA are lost from the telomere ends of the chromosomes, and chromosomal shortening eventually leads to replicative senescence<sup>1</sup>. Telomerase is an enzyme, comprising an RNA component (*i.e.*, hTERC or hTR) and a catalytic component (*i.e.*, hTERT), that is able to restore the DNA base pairs lost from the telomeres during cell division. In cells with active telomerase, chromosomal length is maintained and the cells continue to divide without becoming senescent<sup>1</sup>.

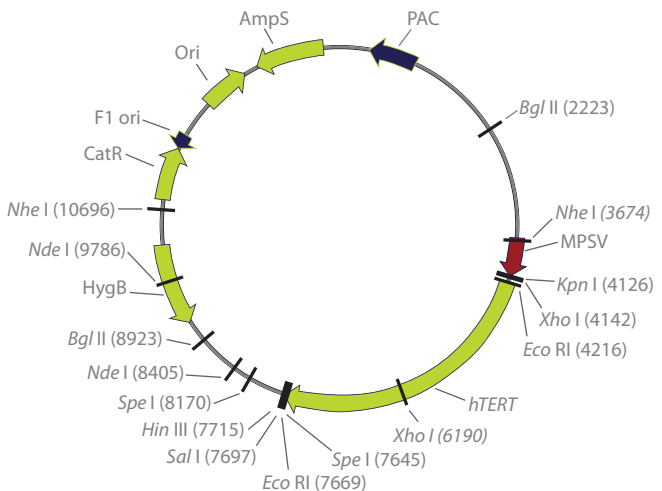
The RNA component of telomerase is expressed ubiquitously<sup>2</sup>, while the expression of the catalytic hTERT component is mainly limited to the early stages of embryonic development, during which time it is expressed by stem cells. In the adult, its expression is restricted to some rare cells of the blood (*i.e.*, white blood cells), germ cells and some cells of the skin and digestive track. Since most normal somatic cells do not have active telomerase, these cells are susceptible to replicative senescence *in vivo* and are difficult to maintain *in vitro*<sup>1</sup>.

## hTERT Immortalization of Primary Cells

Transfection of hTERT into human primary cells leads to elongation and maintenance of the telomere ends of the chromosomes. In many instances, forced expression of hTERT alone enables the cells to repress replicative senescence and overcome the growth crisis, effectively leading to their immortalization<sup>2,3,4</sup>. In some cases, more than one immortalization agent may be required to successfully immortalize a particular cell type. For example primary cell lines may be immortalized using a combination of hTERT with one or more of the following: genes encoding viral (simian virus 40 (SV40) large T antigen<sup>5,6</sup> and human papilloma virus-16 (HPV-16) E6/E7<sup>7,8</sup>) or non-viral (Cdk-4<sup>9</sup> and Bmi-1<sup>10</sup>) oncoproteins.

hTERT immortalized cells are mostly diploid, but may become pseudo-diploid especially at high passage number. In many cases, when cells become pseudo-diploid they still retain most primary cell functions (Table 1). The following is a list of primary human cells that have been established by the forced expression of hTERT alone.

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## Properties of hTERT Immortalized Cells

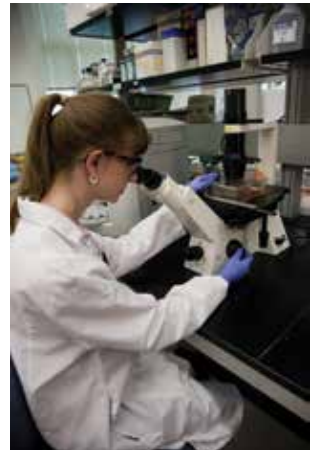
Early-passage hTERT immortalized cells faithfully represent the physiological properties of normal cells *in vivo*. Additionally, analysis of numerous hTERT immortalized cell lines has confirmed that these cells retain the expression of phenotypic markers and have a stable karyotype<sup>13,14</sup>. This is in contrast to many traditional lines of immortalized cells, which are known to develop an unstable karyotype, especially at high passage numbers and when oncogenes are used. Below is a summary of the commonly observed properties of cells immortalized by the ectopic expression of hTERT.

- Nonmalignant<sup>17,19</sup>
- Normal cell cycle controls, functional p53 and pRB checkpoints<sup>17,18</sup>
- Contact inhibited<sup>19</sup>
- Anchorage dependent<sup>19</sup>
- Retain normal growth responses to serum and mitogens<sup>3</sup>
- Require growth factors for proliferation<sup>3</sup>
- Possess a normal karyotype<sup>4,18,\*</sup>
- Do not show changes associated with transformation such as tumorigenicity or growth in soft agar<sup>20</sup>

### Benefits of hTERT immortalized cells

Primary cells closely represent the physiological state of a particular cell type *in vivo*, but they are susceptible to replicative senescence, so their value in the laboratory setting is limited. This is especially true when large quantities of cells are required for biochemical analysis, genetic manipulations or for genetic screens. It is also a factor for the study of some types of rare hereditary human diseases, since the volume of the biological samples collected (biopsies or blood) is usually small and contains a limited number of cells. Continuous cell lines, on the other hand, are not encumbered by replicative senescence, but, they often contain numerous genetic mutations, exhibit an unstable karyotype and have protein expression patterns that are not comparable with the cell type they are intended to represent.

hTERT cells combine the physiological attributes of primary cell lines and the long culture life of continuous cell lines, while avoiding the replicative senescence of the former and the unstable karyotype of the latter. Additionally, in many studies hTERT immortalized cells have been induced to become differentiated cell types, exhibiting tissue-specific features, differentiation-specific proteins, and forming structures that resemble those formed *in vivo*<sup>17</sup>.



\*For Karyotype information please see appendix on page 28.

## Telomeres and Telomerase

Table 1. Comparison Between hTERT Immortalized Cells, Primary Cells, Oncogene/Viral Immortalized Cells and Continuous Cell Lines

	Primary Cells	hTERT-Immortalized	Onco, Viral-Immortalized	Continuous
Mimic <i>in vivo</i> Tissue Phenotype	++++	+++	++	+
Karyotypic Stability	Diploid	Diploid/ Pseudodiploid	Pseudodiploid/ Aneuploid	Aneuploid
Proliferative Capacity	+	+++	+++	+++
Supply	+	+++	+++	+++
Inter-Experimental Reproducibility	Low	Good	Good	Good
Cost	High	Medium	Low	Low
Ease of Use	+	++	++	+++

### Applications

The unique properties of hTERT immortalized cells, as discussed above (and illustrated in table 1), make them an attractive replacement for both primary and transformed cell lines and a valuable tool for the study of cell functions both *in vitro* and *in vivo*. The following is a list of published applications of hTERT immortalized cells.

- Long-term studies of biochemical and physiological aspects of cell growth, *e.g.*, endogenous protein markers, gene expression, and growth inhibition<sup>12</sup>
- *In vitro* model for differentiation and carcinogenesis<sup>7,12</sup>
- Cancer research and studies of oncogenes<sup>7,25</sup>
- Cell based drug screening, and drug toxicity testing<sup>26</sup>
- Tissue engineering, and transplantation<sup>27</sup>
- Genetic engineering and modifications<sup>28</sup>
- Biological functions of hTERT<sup>29</sup>

# ATCC hTERT Immortalized Cell Solutions

ATCC hTERT immortalized cell lines represent a breakthrough in cell biology research that combine the *in vivo* nature of primary cells and the *in vitro* utility of continuous cell lines. Until recently, cell biologists had to choose between primary cells and established cell lines as the basis for their experimental models, but both options had their flaws. Normal primary cells are difficult to isolate, often vary from lot to lot, and senesce after a few passages. Traditional cell lines, on the other hand, are genetically unstable and present inconsistent phenotypes over time. Now, with hTERT immortalized cell lines from ATCC, cell biologists can avoid the limitations, while enjoying the benefits of both.

In addition to the standard ATCC authentication, all ATCC hTERT cell lines are tested for:

- Extended proliferative capacity
- Stable karyotype
- Selected phenotypic markers from the tissue of interest
- Continued expression of hTERT

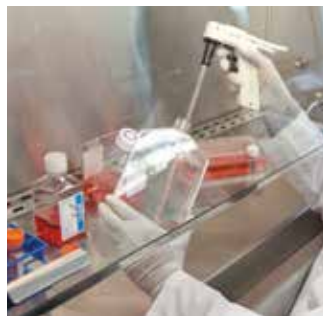
## ATCC hTERT Cell Immortalization Tools

A major obstacle to the immortalization of primary human cells and the establishment of human cell lines is telomere-controlled senescence, caused by the shortening of telomeres that occurs each time somatic human cells divide<sup>1</sup>. The enzyme telomerase can prevent the shortening of telomeres, and the transfer of exogenous hTERT cDNA (encoding the catalytic subunit of human telomerase) can be used to prevent telomere shortening, overcome telomere-controlled senescence, and immortalize primary human cells.

## ATCC hTERT Immortalization Vectors

This set of immortalization products enables researchers to immortalize primary cells of interest in their labs using the hTERT technology.

- hTERT vectors: ATCC® No. MBA-141
- Viral oncoprotein vectors:
  - HPV-16 E6/E7: ATCC® No. 45113™; ATCC® No. 45113D™
- Non-viral (Cdk-4 and Bmi-1) oncoprotein vectors: 81582D



# ATCC hTERT Immortalized Barrett's Esophageal Epithelial Cells

## Introduction

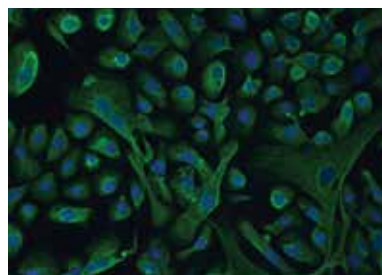
Primary Barrett's epithelial cells have a limited lifespan in culture and typically do not contain the genetic abnormalities that can lead to cancer in Barrett's esophagus, which significantly limits their use as a model for the progression of this disease. In contrast, ATCC hTERT immortalized Barrett's esophageal epithelial cells contain stable, defined cell cycle and genetic abnormalities, have an extended life span, and are karyotypically, morphologically, and phenotypically similar to the primary parent cells.

The Barrett's esophagus cell lines, CP-A (KR-42421), ATCC® No. CRL-4027™, CP-B (CP-52731), ATCC® No. CRL-4028™, CP-C (CP-94251), ATCC® No. CRL-4029™, and CP-D (CP-18821), ATCC® No. CRL-4030™ were derived from an endoscopic biopsy specimen obtained from a region of non-dysplastic metaplasia and transduced with the retroviral expression vector, pLXSN-hTERT<sup>2</sup>.

## Cell Culture Protocols

### Materials Needed

- MCDB-153 medium (Sigma, M7403)
- Hydrocortisone
- Recombinant human epidermal growth factor
- Cholera toxin
- Adenine
- Bovine pituitary extract
- Insulin-transferrin-sodium selenite supplement (Sigma, I1884)
- Glutamine
- Fetal bovine serum, ATCC® No. 30-2020
- Dulbecco's phosphate buffered saline, ATCC® No. 30-2200
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS), ATCC® No. 30-2101
- Erythrosin B vital stain solution
- Cell culture tested DMSO, ATCC® No. 4-X
- RPMI-1640 medium, ATCC® No. 30-2001



ATCC® No. CRL-4027™ stained with a monoclonal pan-cytokeratin antibody (green) and Hoechst dye (blue)

### Preparation of Complete Growth Medium

The base medium for these cell lines is MCDB-153. To make the complete growth medium add the following components to the base medium:

- 0.4 µg/mL hydrocortisone
- 20 ng/mL recombinant human epidermal growth factor
- 1 nM cholera toxin
- 20 mg/L adenine
- 140 µg/mL bovine pituitary extract
- 0.1% insulin-transferrin-sodium selenite supplement
- 4 mM glutamine
- 5% fetal bovine serum



### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and not at -70°C. Storage at -70°C will result in loss of viability.

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC primary Barrett's epithelial cells.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Determine the number of viable cells and add an appropriate aliquot of the suspension to a culture flask.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Subculturing and Maintenance of Cultures

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium required proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. Proceed to the next step when the cells have reached the recommended density and are ready to be subcultured.
3. Remove and discard the media.
4. Rinse the cells with Dulbecco's phosphate buffered saline to remove any trace of serum.
5. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
6. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.  
Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
7. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
8. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

**Note:**

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. To facilitate dispersal of cells place the flask at 37°C.

### Cryopreservation Medium

Freeze cells in the following medium: RPMI-1640 Medium supplemented with 10% fetal bovine serum and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

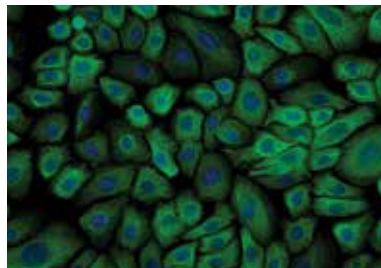
# ATCC hTERT Immortalized Bronchial Epithelial Cells

## Introduction

The use of normal and diseased primary bronchial epithelial cultures is restricted by the limited availability of normal and diseased primary cell pairs, significant inconsistency between donors and their finite proliferative capacity. Moreover, the available cell lines have been transformed using viral genes or derived from tumors and do not maintain the morphology and phenotype of the parent cells. However, ATCC hTERT immortalized bronchial epithelial cells have an extended lifespan, have a stable karyotype and are phenotypically similar to the primary parent cells.

The human airway epithelial cell line, NuLi-1 (ATCC® No. CRL-4011™), was derived from normal lung epithelial cells by dual retroviral infection with HPV-16 E6/E7-LXSN and hTERT-LXSN.

Human airway epithelial cell line, CuFi-1 (ATCC® No. CRL-4013™), CuFi-4 (ATCC® No. CRL-4015™), CuFi-5 (ATCC® No. CRL-4016™), and CuFi-6 (ATCC® No. CRL-4017™) were derived from the lung epithelial cells of cystic fibrosis patients by dual retroviral infection with HPV-16 E6/E7-LXSN and hTERT-LXSN or pBabe-hygro-hTERT<sup>9</sup>.



ATCC® No. CRL-4011™ stained with a monoclonal pan-cytokeratin antibody (green) and Hoechst dye (blue)

## Cell Culture Protocols

### Materials Needed

- Bronchial Epithelial Growth Medium (BEGM), serum-free (Lonza BEGM BulletKit™, CC-3170)
- G-418
- Human placental collagen type IV, (Sigma, C-7521)
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS) ATCC® No. 30-2101
- Erythrosin B vital stain solution
- Cell culture tested DMSO, ATCC® No. 4-X
- Fetal bovine serum, ATCC® No. 30-2020
- Dulbecco's phosphate buffered saline, ATCC® No. 30-2200

### Preparation of Complete Growth Medium

The base medium for these cell lines is BEBM. To make the complete growth medium (BEGM) add the following components to the base medium:

- SingleQuot™ additives (supplied with the BEGM BulletKit™)
- 50 µg/mL G-418

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and **not** at -70°C. Storage at -70°C will result in loss of viability.

#### Note:

ATCC does not use the gentamycin-amphotericin B supplied with the BEGM kit.

The culture flasks should be pre-coated with 60 µg/mL solution of human placental collagen type IV at least 18 hours in advance, then air-dried and rinsed 2 to 3 times with Dulbecco's phosphate buffered saline.

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC primary bronchial epithelial cells.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Subculturing Procedure

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days (do not exceed 3 days).
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Add 2.0 to 3.0 mL trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
5. To remove trypsin-EDTA solution, add 2.0 to 3.0 mL of 1% FBS in Dulbecco's phosphate buffered saline and aspirate cells by gently pipetting.
6. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
7. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.
8. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

#### Note:

The culture flasks should be pre-coated with 60 µg/mL solution of human placental collagen type IV at least 18 hours in advance, then air-dried and rinsed 2 to 3 times with Dulbecco's phosphate buffered saline.

#### Note:

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

### Cryopreservation Medium

Freeze cells in the following medium: BEGM supplemented with 10% DMSO and 30% fetal bovine serum. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Chondrocyte Fibroblast Cells

## Introduction

Primary human fetal chondrocytes exhibit a relatively short life span under standard culture conditions, which severely restricts their ability to be used in research studies. ATCC hTERT immortalized chondrocytes have an extended lifespan, retain expression of chondrocyte specific markers and are karyotypically, morphologically, and phenotypically similar to the primary parent cells.

The chondrocyte cell lines, CHON-001 (ATCC® No. CRL-2846™) and CHON-002 (ATCC® No. CRL-2847™), were derived from chondrocytes of normal human long bones infected by hTERT-LXSN under G-418 selection.

## Cell Culture Protocols

### Materials Needed

- ATCC-formulated Dulbecco's modified Eagle's medium (DMEM), ATCC® No. 30-2002
- G-418
- Fetal bovine serum, ATCC® No. 30-2020
- Dulbecco's phosphate buffered saline ATCC® No. 30-2200
- Trypsin-EDTA Solution (0.05% Trypsin/0.53 mM EDTA, such as Gibco® 25300-054) or Trypsin-EDTA for primary cells (ATCC® No. PCS-999-003)
- Erythrosin B vital stain solution
- Cell culture tested DMSO, ATCC® No. 4-X

### Preparation of Complete Growth Medium

The base medium for these cell lines is ATCC-formulated DMEM. To make the complete growth medium, add the following components to the base medium:

- 0.1 mg/mL G-418
- 10% heat-inactivated fetal bovine serum

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at -70°C. Storage at -70°C will result in loss of viability.

**Note:**

This medium is formulated for use with a 5% CO<sub>2</sub> in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO<sub>2</sub> in air atmosphere is then recommended).

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC primary human fetal chondrocytes.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. 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).

4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Subculturing Procedure

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 days, or as needed.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Rinse the cells with Dulbecco's phosphate buffered saline to remove traces of serum.
5. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
6. To remove trypsin-EDTA solution, add 2.0 to 3.0 mL of 1% FBS in Dulbecco's phosphate buffered saline and aspirate cells by gently pipetting.
7. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
8. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.
9. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

**Note:**

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

### Cryopreservation Medium

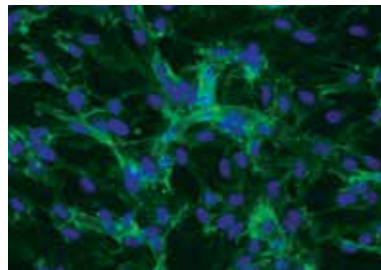
Freeze cells in the following medium: 90% heat-inactivated fetal bovine serum and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Dermal Microvascular Endothelial Cells

## Introduction

ATCC hTERT immortalized dermal microvascular endothelial cells have an extended lifespan, express a panel of endothelial cell surface proteins, undergo tubule formation in culture and are karyotypically, morphologically, and phenotypically similar to the primary parent cells. By overcoming the finite proliferative capacity of primary cells, the hTERT dermal microvascular endothelial cells represent an effective cell model for studying endothelial cell biology including signal transduction and angiogenesis.

The telomerase-immortalized human microvascular endothelium cell line, TIME (ATCC® No. CRL-4025™), was derived from a primary culture of neonatal foreskin microvascular endothelial cells (HMVEC) of the dermis. Primary HMVECs were immortalized by infection with the retrovirus WZLblast3:hTERT. TIME cells express a panel of characteristic endothelial cell surface marker proteins including CD31/PECAM-1 and integrin  $\alpha\text{V}\beta\text{3}$ . The cells also express the low density lipoprotein (LDL) receptor and are capable of acetylated LDL uptake<sup>12</sup>.



ATCC® No. CRL-4025™ stained with a CD31 antibody (green) and Hoechst dye (blue)

## Cell Culture Protocols

### Materials Needed

- Endothelial Cell Basal Medium-2 (EBM-2), (Lonza EGM-2-MV BulletKit™, CC-3202)
- Blasticidine
- Trypsin-EDTA solution (Lonza, CC-5012)
- Trypsin neutralizing solution (Lonza, CC-5002)
- Fetal bovine serum, ATCC® No. 30-2020
- HEPES buffered saline solution (Lonza, CC-5024)
- Erythrosin B vital stain solution
- Cell culture tested DMSO, ATCC® No. 4-X

### Preparation of Complete Growth Medium

The base medium for these cell lines is Endothelial Cell Basal Medium-2 (EBM-2). To make the complete growth medium (EGM-2), add the following components to the base medium:

- SingleQuot™ additives supplied with the kit
- 12.5  $\mu\text{g}/\text{mL}$  blasticidine

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and **not** at  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

#### Note:

ATCC does not use the (gentamycin-amphotericin B mix supplied with the kit.

#### Note:

Do not filter complete medium



## ATCC hTERT Immortalized Dermal Microvascular Endothelial Cells

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC dermal microvascular endothelial cells.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Subculturing Procedure

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made, proceed to the next step.
3. Remove and discard the media.
4. Rinse the cells with room temperature HEPES buffered saline solution.
5. Add 5.0 to 6.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 4 to 8 minutes).
6. To inactivate trypsin-EDTA solution, add 5.0 to 6.0 mL of trypsin neutralizing solution and aspirate cells by gently pipetting.
7. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
8. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.
9. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

**Note:**

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

### Cryopreservation Medium

Freeze cells in the following medium: 90% fetal bovine serum and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Endometrial Fibroblasts

## Introduction

ATCC hTERT immortalized endometrial fibroblasts (T HESCs) have an extended lifespan and are karyotypically, morphologically, and phenotypically similar to the primary parent cells. Functionally, T HESCs display the biochemical endpoints of decidualization after hormone treatment.

T HESCs (ATCC® No. CRL-4003™) were derived from stromal cells obtained from an adult female with myomas. The primary stromal endometrium cells were immortalized by infection with supernatant from the packaging cell line pA317-hTERT which expressed the hTERT and the puromycin resistance genes<sup>23</sup>.

## Cell Culture Protocols

### Materials Needed

- Dulbecco's modified Eagle's medium (DMEM)/F12 (Sigma, D2906)
- Sodium bicarbonate
- ITS+ Universal Culture Supplement Premix (BD, 354352)
- Puromycin
- Charcoal/dextran treated fetal bovine serum (Hyclone, SH30068.03)
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS) ATCC® No. 30-2101
- Cell culture tested DMSO, ATCC® No. 4-X
- Erythrosin B vital stain solution

### Preparation of Complete Growth Medium

The base medium for these cell lines is DMEM/F12 supplemented with:

- 1.5 g/L sodium bicarbonate
- 1% ITS+ Universal Culture Supplement Premix
- 500 ng/mL puromycin
- 10% charcoal/dextran-treated fetal bovine serum

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at -70°C. Storage at -70°C will result in loss of viability.

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC endometrial fibroblasts cells.
2. Prepare a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.





3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 6.0 to 8.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Subculturing Procedure

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made, proceed to the next step.
3. Remove and discard the media.
4. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 15 minutes).
5. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
6. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
7. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.

**Note:**

To avoid clumping do not hit or shake the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

### Cryopreservation Medium

Freeze cells in the following medium: 95% complete growth media, 5% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Human Foreskin Fibroblasts

## Introduction

Human foreskin fibroblasts were among the first primary cell types to be successfully immortalized using hTERT technology, so the functions and properties of hTERT immortalized fibroblasts have been thoroughly characterized. ATCC hTERT immortalized foreskin fibroblasts (BJ-5ta) have an extended lifespan, express PDGFR $\beta$  and are karyotypically, morphologically, and phenotypically similar to the primary parent cells. BJ 5ta are widely used as *in vitro* models for wound healing, tissue engineering and regeneration applications.

The hTERT immortalized foreskin fibroblast cell line, BJ-5ta (ATCC<sup>®</sup> No. CRL-4001™), was derived by transfecting the BJ foreskin fibroblast cell line, at population doubling (PDL) 58, with pGRN145 hTERT followed by selection with hygromycin B<sup>25</sup>.

## Cell Culture Protocols

### Materials Needed

- Dulbecco's Modified Eagle's Medium (DMEM) ATCC<sup>®</sup> No. 30-2002
- M199 medium
- Hygromycin B
- Fetal bovine serum
- Dulbecco's phosphate buffered saline ATCC<sup>®</sup> No. 30-2200
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS), ATCC<sup>®</sup> No. 30-2101

### Preparation of Complete Growth Medium

The base medium for this cell line is a 4:1 mixture of DMEM and M199. To make the complete growth medium add the following components to the base medium:

- 0.01 mg/mL hygromycin B
- 10% fetal bovine serum

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at -70°C. Storage at -70°C will result in loss of viability.

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of human foreskin fibroblasts.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.



3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

## Subculturing and Maintenance of Cultures

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Rinse the cells with Dulbecco's phosphate buffered saline to remove traces of serum.
5. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
6. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
7. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
8. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.
9. Incubate the cultures in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Note:

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

## Cryopreservation Medium

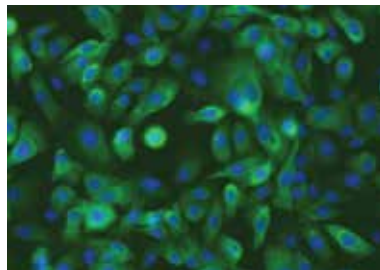
Freeze cells in the following medium: 90% fetal bovine serum and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Mammary Epithelial Cells

## Introduction

ATCC hTERT immortalized mammary epithelial cells (hTERT-HME1) have an extended lifespan, do not express estrogen receptor and are karyotypically, morphologically, and phenotypically similar to the primary parent cells. In addition, these cells retain many differentiated features of normal HMECs. As such, hTERT-HME1 can serve as valuable *in vitro* models to study the stages of breast cancer development.

The human mammary epithelium, HME1 (ATCC® No. CRL-4010™) cell line was derived from normal primary mammary epithelial cells infected with the retrovirus pBabepuro+hTERT vector and cultured in complete growth medium containing puromycin until stable clones were selected<sup>13</sup>.



ATCC® No. CRL-4010™ stained with a monoclonal pan-cytokeratin antibody (green) and Hoechst dye (blue)

## Cell Culture Protocols

### Materials Needed

- Mammary Epithelial Growth Medium, serum-free (MEGM) from Clonetics (Lonza MEGM BulletKit™, CC-3150)
- Soybean trypsin inhibitor, ATCC® No. 30-2104
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS), ATCC® No. 30-2101
- Erythrosin B vital stain solution
- Dulbecco's phosphate buffered saline, ATCC® No. 30-2200

### Preparation of Complete Growth Medium

The base medium for these cell lines is MEBM. To make the complete growth medium (MEGM) add the following components to the base medium:

- SingleQuot™ additives (supplied with the MEGM BulletKit™)

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at -70°C. Storage at -70°C will result in loss of viability.

**Note:**

ATCC does not use the gentamycin-amphotericin B mix provided with the kit. Do not filter complete medium

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC immortalized mammary epithelial cells.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.

3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Subculturing and Maintenance of Cultures

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Add 2.0 to 3.0 mL of soybean trypsin inhibitor solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
5. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
6. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
7. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.

**Note:**

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

### Cryopreservation Medium

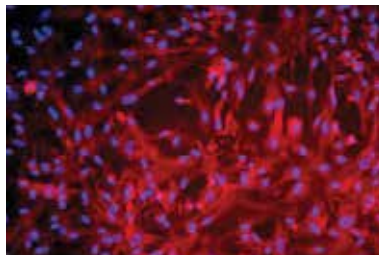
Freeze cells in the following medium: 90% complete culture medium supplemented and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Pancreas Duct Epithelial Cells

## Introduction

Investigating the roles played by cells expressing the neuronal stem cell marker nestin in the human pancreas is important in pancreatic cancer and metabolic disease studies. ATCC hTERT immortalized pancreas duct epithelial cells have an extended lifespan, express nestin, and are karyotypically, morphologically, and phenotypically similar to the primary parent cells.

hTERT-HPNE (Human Pancreatic Nestin Expressing cells) (ATCC® No. CRL-4023™) was developed from human pancreatic duct cells by transduction with a retroviral expression vector (pBABEpuro) containing the hTERT gene. hTERT-HPNE E6/E7 (ATCC® No. CRL-4036™) cells were derived from hTERT-HPNE cells (ATCC® No. CRL-4023™) by infection with retroviral vector pLEXSN carrying HPV-16 E6/E7. hTERT-HPNE E6/E7/st cells were derived from hTERT-HPNE E6/E7 cells (ATCC® No. CRL-4036™) by infection with retroviral vector pBabeZeo carrying the SV40 small T-antigen. hTERT-HPNE E6/E7/K-RasG12D cells were derived from hTERT-HPNE E6/E7 cells (ATCC® No. CRL-4036™) by infection with retroviral vector pLXSN carrying a G12D mutant of the isoform b of human K-Ras. hTERT-HPNE E6/E7/K-RasG12D/st cells were derived from hTERT-HPNE E6/E7/K-RasG12D cells (ATCC® No. CRL-4038™) by infection with retroviral vector pBabeZeo carrying the SV40 small T-antigen<sup>7</sup>.



ATCC® No. CRL-4023™ stained with a monoclonal pan-cytokeratin antibody (red) and Hoechst dye (blue)

## Cell Culture Protocols

### Materials Needed

- DMEM without glucose (Sigma D-5030 with additional 2 mM L-glutamine and 1.5 g/L sodium bicarbonate)
- Medium M3 Base (Incell Corp. M300F- 500)
- Recombinant human epidermal growth factor
- D-glucose
- Puromycin
- Cell culture tested DMSO, ATCC® No. 4-X
- Fetal bovine serum, ATCC® No. 30-2020
- Dulbecco's phosphate buffered saline, ATCC® No. 30-2200
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS), ATCC® No. 30-2101
- Erythrosin B vital stain solution
- Fetal bovine serum, ATCC® No. 30-2020

### Preparation of Complete Growth Medium

The base medium for this cell line is:

- 75% DMEM without glucose
- 25% Medium M3 Base

To make the complete growth medium, add the following components to the base medium:

- Fetal bovine serum to a final concentration of 5%
- 10 ng/mL human recombinant EGF
- 5.5 mM D-glucose (1g/L)
- 750 ng/mL puromycin

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC pancreas duct epithelial cells.
2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately  $125 \times g$  for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a  $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified incubator.

### Subculturing and Maintenance of Cultures

Volumes are given for a  $75 \text{ cm}^2$  flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Rinse the cells with Dulbecco's phosphate buffered saline.
5. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
6. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
7. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately  $125 \times g$  for 5 to 10 minutes.
8. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the

# ATCC hTERT Immortalized Pancreas Duct Epithelial Cells



cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.

9. Incubate cultures in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

## **Cryopreservation Medium**

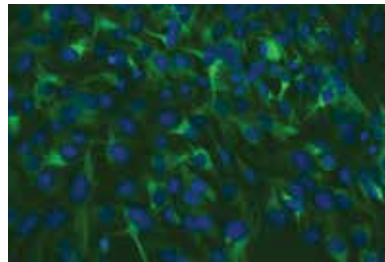
Freeze cells in the following medium: 90% fetal bovine serum and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.



# ATCC hTERT Immortalized Renal Epithelial Cells

## Introduction

Angiomyolipomas are benign tumors of the kidney, which originate from putative perivascular epithelioid cells. These cells may undergo differentiation into cells with features of melanocytes, smooth muscle or fat cells. However, the study of angiomyolipomas is limited by a lack of established angiomyolipoma-derived cell lines and a lack of good animal models. ATCC hTERT immortalized renal epithelial cells are derived from angiomyolipomas, so researchers can now take the advantage of both the primary cell characteristics and extended lifespan of these cells *in vitro*.



ATCC® No. CRL-4004™ stained with a monoclonal pan-cytokeratin antibody (green) and Hoechst dye (blue)

The UMB1949 cell line (ATCC® No. CRL-4004™) expresses NG2 and L1 and has a defined 5bp deletion in exon 33 of tuberin (Tsc2) and mutations in tuberin (and/or hamartin). As such, this cell line can be used to study signal transduction and drug efficiency in tuberous sclerosis. The SV7tert PDGFtu1 (ATCC® No. CRL-4008™) cell line is derived from the tumors caused by the SV7tert implantation in nude mice. The SV7tert cell line is a non-tumorigenic angiomyolipoma cell line immortalized with the SV40 large T antigen and human telomerase, by transduction with a retrovirus encoding PDGF-BB. The tumor-derived cells secrete over 18-fold more PDGF than pre-implantation cells, and demonstrate both autocrine transformation and epigenetic changes<sup>5</sup>.

## Cell Culture Protocols

### Materials Needed

- Dulbecco's Modified Eagle's Medium (DMEM), ATCC® No. 30-2002
- Fetal bovine serum, ATCC® No. 30-2020
- Dulbecco's phosphate buffered saline, ATCC® No. 30-2200
- Trypsin-EDTA Solution (0.25% Trypsin/0.53 mM EDTA in HBSS), ATCC® No. 30-2101
- Erythrosin B vital stain solution
- Cell culture tested DMSO, ATCC® No. 4-X

### Preparation of Complete Growth Medium

The base medium for these cell lines is DMEM. To make the complete growth medium add the following components to the base medium:

- Fetal bovine serum to a final concentration of 10%.

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at -70°C. Storage at -70°C will result in loss of viability.

1. Refer to the batch specific information provided on the last page of the product information sheet for

the total number of viable cells recovered from each lot of ATCC renal epithelial cells.

2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

## Subculturing and Maintenance of Cultures

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Rinse the cells with Dulbecco's Phosphate Buffered Saline to remove traces of serum.
5. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
6. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
7. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
8. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.
9. Incubate the cultures in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Note:

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

## Cryopreservation Medium

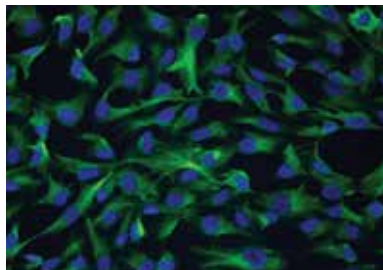
Freeze cells in the following medium: 95% complete growth medium, 5% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# ATCC hTERT Immortalized Retinal Pigmented Epithelial Cells

## Introduction

Retinal pigment epithelium (RPE) is the layer of cells at the base of the retina composed of a single layer of hexagonal cells that are densely packed with pigment granules. The RPE protects and supplies nutrition to the retina, and degeneration of the RPE is linked to age-related macular degeneration (AMD). The hTERT immortalized RPE cell line is a valuable tool in AMD research, with application to studies of RPE functions, disease progression, RPE regeneration, and RPE wound healing.

The hTERT immortalized retinal pigmented epithelial cell line hTERT RPE-1 (ATCC® No. CRL-4000™) was derived by transfecting the RPE-340 cell line with the pGRN145 hTERT-expressing plasmid (ATCC® No. MBA-141). Cells were cultured in medium containing hygromycin B until stable clones were selected<sup>11</sup>.



ATCC® No. CRL-4000™ stained with a monoclonal pan-cytokeratin antibody (green) and Hoechst dye (blue)

## Cell Culture Protocols

### Materials Needed

- Dulbecco's Modified Eagle's Medium (DMEM)/ F12, ATCC® No. 30-2006
- Fetal bovine serum, ATCC® No. 30-2020
- Hygromycin B
- Hanks' balanced salt solution, ATCC® No. 30-2213
- Trypsin-EDTA Solution, ATCC® No. 30-2101
- Cell culture tested DMSO, ATCC® No. 4-X
- Erythrosin B vital stain solution

### Preparation of Complete Growth Medium

The base medium for this cell line is ATCC-formulated DMEM: F12. To make the complete growth medium, add the following components to the base medium:

- Fetal bovine serum to a final concentration of 10%
- 0.01 mg/mL hygromycin B

### Handling Procedure for Frozen Cells and Initiation of Cultures

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor and **not** at -70°C. Storage at -70°C will result in loss of viability.

1. Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from each lot of ATCC retinal pigmented epithelial cells.

2. Prepare a culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
3. 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).
4. 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 operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
6. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
7. Incubate the culture in a 37°C, 5% CO<sub>2</sub>, humidified incubator.

## Subculturing and Maintenance of Cultures

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Maintain the cells in culture by refreshing the medium every 2 to 3 days.
2. Refer to the product information sheet for the concentration at which cells should be subcultured. When a determination that the cells are ready to be subcultured is made proceed to the next step.
3. Remove and discard the media.
4. Rinse with Hanks' balanced salt solution to remove traces of serum.
5. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes).
6. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
7. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
8. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Refer to the product sheet for the recommended inoculum concentration.
9. Incubate cultures at 37°C, 5% CO<sub>2</sub>, humidified incubator.

### Note:

To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.

## Cryopreservation Medium

Freeze cells in the following medium: 30% culture medium, 60% fetal bovine serum and 10% DMSO. Store vials in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

# Appendix

## hTERT Karyotype Information

ATCC® No.	Karyotype
CRL-2846™	This is a diploid cell line of female origin. Overall, the karyology is stable with a modal chromosome number of 46 in 93% of the examined cells and a low rate of polyploidy. No consistent structural chromosomal aberrations were found in any of the cells examined.
CRL-2847™	This is a diploid cell line of female origin. Overall, the karyology is stable with a modal chromosome number of 46 in 87% of the examined cells and a low rate of polyploidy. No consistent structural chromosomal aberrations were found in any of the cells examined.
CRL-4000™	This is a near-diploid human cell line of female origin with a modal chromosome number of 46 that occurred in 90% of the cells counted. The sex chromosomes consist of a karyotypically normal X-chromosome and a derivative X-chromosome with additional chromosomal material at the terminal end of the q-arm. The derivative X-chromosome was present in all of the cells analyzed.
CRL-4001™	This is a diploid human cell line of male origin with a modal chromosome number of 46 that occurred in 90% of the cells counted. The sex chromosomes, X and Y are both karyotypically normal.
CRL-4003™	This is a diploid cell line of female origin. Overall, the karyology is stable with a modal chromosome number of 46 in 57% of the examined cells and a low rate of polyploidy. No consistent structural chromosomal aberrations were found in any of the cells examined.
CRL-4004™	This cell line is of male origin and 1/2 to 2/3 of the total cell population is pseudodiploid, the rest of the cells fall in the tetraploid range. Consistent cytogenetic changes include chromosome 10 and 19 aberration, and chromosome 4 monosomy. Some cells showed loss of the Y chromosome and many of the examined cells contained random chromosomal aberrations.
CRL-4008™	This is a hypotetraploid cell line with many structural rearrangements, numerical losses and gains. The following eight derivatives were found to be present in low and high passage karyotypes: der(x)t(X;3)(q28;p21), der(1)t(1;17)(q10;p10), der(3)t(3;6)(p10;p10), i(8)(q10), i(12)(q10), der(13)t(13;21)(q10;q10), der(16)t(4;16)(q21;q24), add(20)(q13.3). Generally, the karyotyped passages contained the same complement of chromosome rearrangements, losses and gains.
CRL-4010™	This is a pseudo-diploid cell line of female origin with a modal chromosome count of 46 and a low-to-moderate rate of polyploidy. However, even though the line generally has 46 chromosomes per cell, several of those 46 were derivative or marker chromosomes. There were two copies of a karyotypically normal X-chromosome present in 50-60% of the cells. Other features included a normal variation in the heterochromatic region of chromosome 1 (1qh+), a consistent derivative-10 marker chromosome (present in most cells) and 2 other markers: del(3)(p24?) and del(16)(q21~23?) (present in approximately 20-30% of the analyzed cells). Overall, approximately 3-8 marker chromosomes were present in the analyzed metaphase spreads and satellite associations appeared sporadically.
CRL-4011™	This is a near-diploid human cell line of male origin with a polyploidy rate of 24%. There were copies of karyotypically normal X and Y-chromosomes present in most of the cells analyzed. Overall, some of the cells contained chromosomal abnormalities, with the most consistent being trisomy 5 and 20.
CRL-4013™	This is a near-diploid human cell line of female origin with a modal chromosome count of 46 and a polyploidy rate of 27%. There were two copies of a karyotypically normal X-chromosome present in most of the cells. Overall, some of the cells contained chromosomal abnormalities, with most consistent being trisomy 20.
CRL-4015™	The karyotypes of several different passages were determined. This is a human cell line of female origin, and the ploidies range from near-diploid to near-tetraploid. The karyology seems to stabilize at higher passages in the hyperdiploid range with trisomies or tetrasomies of chromosomes 1, 5, 8, 11 and 20. Additional copies of chromosomes 5 and 20 were the most consistent aberrations found throughout all the passages and ploidies.
CRL-4016™	This is a near-diploid cell line of male origin in which the most consistent karyotypic aberrations are trisomy of chromosomes 5 and 20. Other non-clonal aberrations were found at early passage, but the karyology tended to stabilize within several passages.

### hTERT Karyotype Information (continued)

ATCC® No.	Karyotype
CRL-4017™	This is a near-diploid cell line of male origin. The most consistent karyotypic aberrations include trisomy of chromosome 5 and 20, monosomy of chromosome 15 or 16, and loss of the Y chromosome. Additionally, the polyploidy rate may increase slightly at high passage.
CRL-4023™	This is a pseudodiploid human cell line of male origin with a modal chromosome number of 46 and a low polyploidy rate. Approximately 50% of the cells contained a consistent derivative chromosome 21 with additional material at p12.
CRL-4025™	This is a diploid cell line of male origin with a modal chromosome number of 46 and a low rate of polyploidy. The line shows some karyotypic instability at later passages.
CRL-4027™	This is a near-diploid cell line of male origin in which 2 sub-clones make up the majority of the cell population. One clone containing i(8)(q10) and trisomy 20 and the other containing der(1)t(1;18)(q10;q10), i(8)(q10), der(13)t(13;22)(q10;q10) and trisomy 20. The remaining population is generally made up of cells with non-clonal aberrations that were derived from the two major clones. Also, the non-clonal cell population may increase at high passages.
CRL-4028™	This is a hypodiploid cell line of male origin with the following derivative chromosomes consistently present at different passages: der(1)t(1;17)(q42;q21), add(8)(p11.2), der(9)t(9;14)(q10;q10), add(12)(q13), add(15)(q24.3), add(17)(p11.2), del(19)(p13.1), del(21)(q22.1). It should be noted that the tetraploid population was essentially a duplicate of the hypodiploid population and may range from around 18% at lower passages to as high as 50% at higher passages.
CRL-4029™	Genetic instability studies using flow cytometry and FISH reveal the retention of elevated tetraploidy (G2/tetraploidy) in the hTERT-immortalized cells similar to the non-transduced parental cells.
CRL-4030™	This is a hypotetraploid human cell line with the following derivative chromosomes consistently present at several different passages: add(2)(q13), der(3)t(3;8)(p10;q10), ider(7)(q10)dup(q31), der(12)t(12;13)(p10;q10), der(14)t(14;15)(q10;q10), der(15)t(15;22)(q10;q10), add(22)(q13)x2. In addition, there were consistent losses of one copy of chromosomes X, 10, 13, 14, 15, 19 and 20. Other less consistent structural aberrations were observed in some of the examined cells.
CRL-4036™	This is a cell line of male origin that contains two major clonal cell populations: 45-47, XY,der(21)t(17;21)(q21.3;p13) and 46,XY, t(3;18) (p21.1;q11.2),der(21)t(17;21)(q21.3;p13). Other chromosomal aberrations were observed in the examined cells of both clones, but none were of a consistent nature.
CRL-4037™	This is a pseudodiploid human cell line of male origin with a der(21)t(17;21)(q21.3;p13). Another subclone, present at earlier passages, may contain the additional derivative chromosome: der(3)t(3;18)(p21.1;q11.2). Overall, the cell line has a relatively stable karyotype.
CRL-4038™	This is a pseudodiploid human cell line of male origin. Clonal aberrations included the derivative chromosomes: t(3;18)(p21;q11.2) [balanced translocation], del(6)(q15), add(8)(q11.2) and der(21)t(17;21)(q21;p11.2). The percentage of cells with the normal male chromosome complement increased at high passage and non-clonal aberrations were seen in approximately 20% of the examined cells at all passages.
CRL-4039™	This is a human cell line of male origin with three major clonal cell populations: 45-48,XY,t(3;18)(p21;q11.2),der(21)t(17;21)(q21;p11.2), 47-48,idem,+20 and 45-48,XY, der(21)t(17;21)(q21;p11.2). Other non-clonal, chromosomal aberrations may be present in the cells of the three major clones.

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Tissue Type	hTERT Immortalized Cells	ATCC® No.	Designations	Depositor	Immortalization Method
Breast	hTERT human mammary epithelium	CRL-4010™	hTERT-HME1	JW Shay, U Texas-SW	pBabepuro+hTERT
Bone	hTERT human bone cartilage fibroblast	CRL-2846™	CHON-001	Y Reid, ATCC	pLXSN-hTERT
Bone	hTERT human bone cartilage fibroblast	CRL-2847™	CHON-002	Y Reid, ATCC	pLXSN-hTERT
Esophagus	hTERT Barrett's esophageal epithelial cells	CRL-4027™	CP-A(KR-42421)	B Reid, U Washington - Seattle	pLXSN-hTERT
Esophagus	hTERT Barrett's esophageal epithelial cells	CRL-4028™	CP-B(CP-52731)	B Reid, U Washington - Seattle	pLXSN-hTERT
Esophagus	hTERT Barrett's esophageal epithelial cells	CRL-4029™	CP-C(CP-94251)	B Reid, U Washington - Seattle	pLXSN-hTERT
Esophagus	hTERT Barrett's esophageal epithelial cells	CRL-4030™	CP-D(CP-18821)	B Reid, U Washington - Seattle	pLXSN-hTERT
Eye	hTERT retinal pigmented epithelial cells	CRL-4000™	hTERT RPE-1	Geron Corp.	pGRN145 hTERT
Kidney	hTERT human renal epithelium, tuberous sclerosis, angiomyolipoma	CRL-4004™	UMB1949	JL Arbiser, Emory U	hTERT+SV40
Kidney	hTERT human renal epithelium, tuberous sclerosis, angiomyolipoma	CRL-4008™	SV7tert PDGF tumor-1	JL Arbiser, Emory U	hTERT+SV40+PDGF-BB
Lung	hTERT human bronchial epithelium, normal	CRL-4011™	NuLi-1	AJ Klingelhut, U Iowa	pLXSN-hTERT, HPV-16E6/E7-LXSN
Lung	hTERT human bronchial epithelium, cystic fibrosis	CRL-4013™	CuFi-1	AJ Klingelhut, U Iowa	pLXSN-hTERT, HPV-16E6/E7-LXSN
Lung	hTERT human bronchial epithelium, cystic fibrosis	CRL-4015™	CuFi-4	AJ Klingelhut, U Iowa	pLXSN-hTERT, HPV-16E6/E7-LXSN
Lung	hTERT human bronchial epithelium, cystic fibrosis	CRL-4016™	CuFi-5	AJ Klingelhut, U Iowa	pLXSN-hTERT, HPV-16E6/E7-LXSN
Lung	hTERT human bronchial epithelium, cystic fibrosis	CRL-4017™	CuFi-6	AJ Klingelhut, U Iowa	pLXSN-hTERT, HPV-16E6/E7-LXSN
Pancreas	hTERT human normal pancreatic duct epithelium	CRL-4023™	hTERT-HPNE	M Ouellette, U Nebraska	pBabepuro+hTERT
Pancreas	hTERT human pancreatic duct epithelium	CRL-4036™	hTERT-HPNE E6/E7	M Ouellette, U Nebraska	derived from CRL-4023 + pLEXSN-HP
Pancreas	hTERT human pancreatic duct epithelium	CRL-4037™	hTERT-HPNE E6/E7/st	M Ouellette, U Nebraska	derived from CRL-4036 + pBabeZeo-
Pancreas	hTERT human pancreatic duct epithelium	CRL-4038™	hTERT-HPNE E6/E7/K-RasG12D	M Ouellette, U Nebraska	derived from CRL-4036 + pLXSN-K-R
Pancreas	hTERT human pancreatic duct epithelium	CRL-4039™	hTERT-HPNE E6/E7/K-RasG12D/st	M Ouellette, U Nebraska	derived from CRL-4038 + pBabeZeo-
Skin	hTERT human dermal microvascular endothelium	CRL-4025™	TIME	M McMahan, UCSF	WZLblast3:hTERT
Skin	hTERT human foreskin fibroblast	CRL-4001™	BJ-5ta	Geron Corp.	pGRN145 hTERT
Uterus	hTERT human endometrium fibroblast	CRL-4003™	T HESCs	GG Mor, Yale U (G.Kirkun on the product datasheet)	pA317-hTERT

	Growth Medium	PubMed		Applications
		(PMID)	Date	
	Lonza MEGM	12420227	2002	1. Long-term studies of biochemical and physiological aspects of cell growth; 2. In vitro model for differentiation and carcinogenesis; 3. Cancer research and studies of oncogenes; 4. Genetic engineering and modifications; 5. Tissue engineering, and transplantation; 6. Gene expression studies; 7. Cell based drug screening, and drug toxicity testing; 8. Biological functions of the hTERT
	ATCC® DMEM + 10% heat-inactivated ATCC® FBS, 0.1mg/mL G-418		2001	
	ATCC® DMEM + 10% heat-inactivated ATCC® FBS, 0.1mg/mL G-418		2001	
	MCDB-153 +: 0.4 µg/mL hydrocortisone; 20 ng/mL human EGF; 1 nM cholera toxin; 20 mg/L adenine; 140 µg/mL BPE; 0.1% ITS; 4 mM ATCC® glutamine; 5% ATCC® FBS	9453489	1995	
	MCDB-153 +: 0.4 µg/mL hydrocortisone; 20 ng/mL human EGF; 1 nM cholera toxin; 20 mg/L adenine; 140 µg/mL BPE; 0.1% ITS; 4 mM ATCC® glutamine; 5% ATCC® FBS		1995	
	MCDB-153 +: 0.4 µg/mL hydrocortisone; 20 ng/mL human EGF; 1 nM cholera toxin; 20 mg/L adenine; 140 µg/mL BPE; 0.1% ITS; 4 mM ATCC® glutamine; 5% ATCC® FBS		1995	
	MCDB-153 +: 0.4 µg/mL hydrocortisone; 20 ng/mL human EGF; 1 nM cholera toxin; 20 mg/L adenine; 140 µg/mL BPE; 0.1% ITS; 4 mM ATCC® glutamine; 5% ATCC® FBS		1995	
	ATCC® DMEM:F12 + 10% ATCC® FBS + 0.01 mg/mL hygromycin B	11980883	2002	
	ATCC® DMEM + 10% ATCC® FBS	17592550	2007	
	ATCC® DMEM + 10% ATCC® FBS	15695519	2003	
	Lonza BEGM	12676769	2002	
	Lonza BEGM	12676769	2002	
	Lonza BEGM	12676769	2002	
	Lonza BEGM	12676769	2002	
	Lonza BEGM	12676769	2002	
	ATCC® DMEM:M3 (3:1) +: 5% ATCC® FBS; 10 ng/mL human EGF; 5.5 mM D-glucose (1g/L); 750 ng/mL puromycin	12589817	2003	
SV16E6/E7	ATCC® DMEM:M3 (3:1) + 5% ATCC® FBS; 10 ng/mL human EGF; 5.5 mM D-glucose (1g/L); 750 ng/mL puromycin	17332339	2007	
SV40st	ATCC® DMEM:M3 (3:1) + 5% ATCC® FBS; 10 ng/mL human EGF; 5.5 mM D-glucose (1g/L); 750 ng/mL puromycin	17332339	2007	
asG12D	ATCC® DMEM:M3 (3:1) + 5% ATCC® FBS; 10 ng/mL human EGF; 5.5 mM D-glucose (1g/L); 750 ng/mL puromycin	17332339	2007	
Sv40st	ATCC® DMEM:M3 (3:1) + 5% ATCC® FBS; 10 ng/mL human EGF; 5.5 mM D-glucose (1g/L); 750 ng/mL puromycin	17332339	2007	
	Lonza EGM-2	11795943	2001	
	ATCC® DMEM:M199 (4:1) +: 0.01 mg/mL hygromycin B; 10% ATCC® FBS	9454332	1998	
	ATCC® DMEM:F12 + : 1% ITS+ Premix (BD), 500ng/mL puromycin, 10%; charcoal treated ATCC® FBS	14726435	2004	

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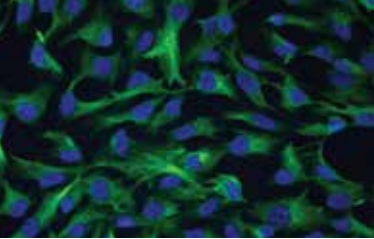
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