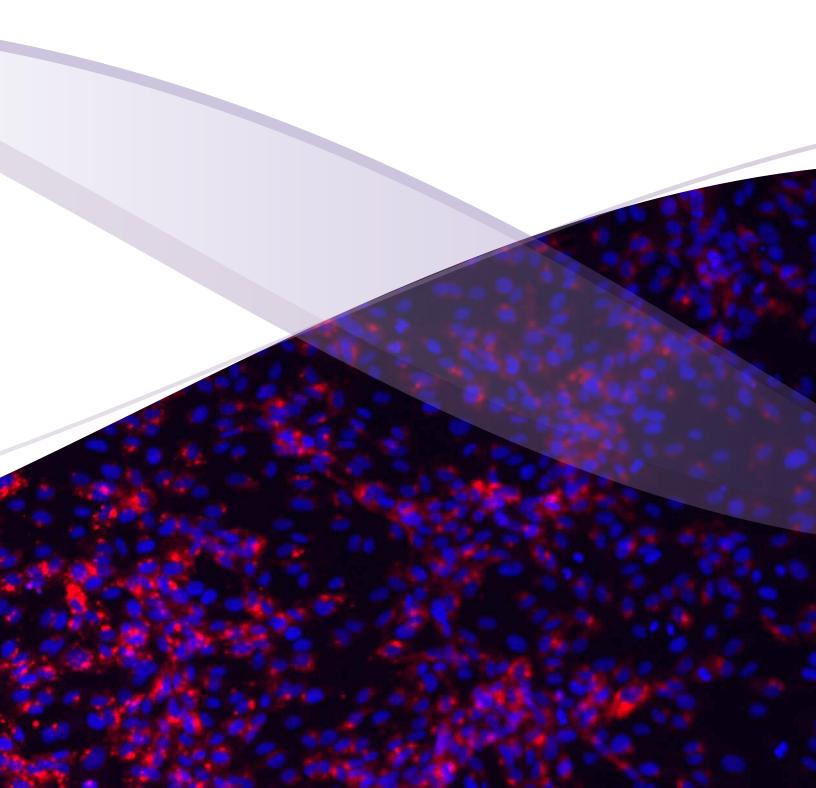


Primary Cell Solutions



Primary Cells are isolated directly from tissue and thus provide a physiologically relevant accompaniment to continuous cell lines; in some cases, they may be a preferred model. Unlike continuous cell lines, primary cells are only maintained for a limited time, so the potential for genetic and phenotypic drift is reduced while their resemblance to the in vivo state is preserved.

The ATCC Primary Cell Collection includes an array of cell types derived from a variety of different tissues. These cells represent powerful experimental platforms that researchers can use to explore cell biology in new and meaningful ways. We hope you find this guide helpful for selecting the cells and media that you need to get your experiments up, running and moving towards your research goals.

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ATCC provides research and development tools and reagents as well as related biological material management services, consistent with its mission: to acquire, authenticate, preserve, develop, and distribute standard reference microorganisms, cell lines, and related materials for research in the life sciences.

For over 95 years, ATCC has been a leading provider of high-quality biological materials and standards to the life science community. We are an independent, 501(c)(3) non-profit entity focused on scientific enablement at universities, research institutes, government agencies, and commercial research labs. Our diverse and comprehensive resources in cell biology and microbiology have been central to the growth of the biotechnology age. ATCC has as its core mission to source, authenticate and further develop products and services essential to the needs of basic and applied life science work.

ATCC distributes to more than 165 countries on 6 continents and has a growing international network of 15 distribution partners. Our infrastructure and experience in biological materials logistics enables us to work effectively with researchers no matter where they are located.

AIRWAY CELLS

Each lot of ATCC Normal Human Pulmonary Primary Cells is:

- Cryopreserved in the first or second passage to ensure the highest viability and plating efficiency
- Performance tested with ATCC Primary Cell Solutions media, kit supplements and reagents to guarantee optimum reliability
- Thoroughly tested for sample purity as part of the ATCC commitment to quality The ATCC collection of primary cells includes cells isolated from respiratory tissue.

RESPIRATORY EPITHELIAL CELLS

- Small Airway Epithelial Cells, Normal, Human ATCC[®] <u>PCS-301-010</u>[™]
- Bronchial/Tracheal Epithelial Cells, Normal, Human ATCC[®] <u>PCS-300-010</u>[™]

These are among the most widely used in vitro models for research related to:

- Asthma, airway inflammation and wound healing
- Pulmonary fibrosis; COPD, including chronic bronchitis and emphysema
- Microbial infection and pathogenesis, including influenza
- Cancer
- Toxicology

OPTIMIZED MEDIA SOLUTIONS

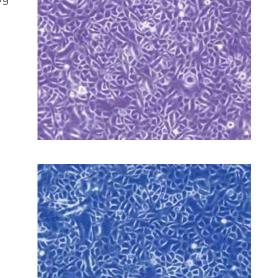
Airway Epithelial Cell Basal Medium is a sterile, phenol red-free, liquid tissue culture medium intended for use as one component in a complete ATCC Primary Cell Solutions system. Used in combination with the Bronchial Epithelial Cell Growth Kit the complete ATCC Primary Cell Solutions airway epithelial cell system selectively sustains the proliferation and plating efficiency of cells derived from normal human bronchial/tracheal tissues or bronchioles. Use of

this complete system removes the need for additional components such as feeder layers, extracellular matrix proteins or other substrates.

- Small Airway Epithelial Growth Kit ATCC[®] PCS-301-040[™]
- Bronchial Epithelial Growth Kit ATCC[®] <u>PCS-300-040</u>[™]

Growth Characteristics of Primary Airway Epithelial Cells in ATCC Complete Growth Medium

Cell Type	Number of Doublings	Average Doubling Time (hrs)	Average Doubling/Passage
Bronchial/Tracheal Epithelial Cells	13.6	28.0	3.4
Small Airway Epithelial Cells	15.4	33.3	3.3



Growth of ATCC Primary Cell Solutions Primary Airway Epithelial Cells in Different Brands of Serum-Free Media



Figure 1: ATCC Primary Cell Solutions airway epithelial cells were taken from liquid nitrogen and cultures initiated. The cells were cultured for 3 to 4 days. Bronchial/tracheal epithelial cells were then seeded in triplicate into a 24-well plate at 1,000 cells/cm², 500 cells/cm², or 250 cells/cm², and small airway epithelial cells were seeded at 2,000 cells/cm², 1,000 cells/cm², or 400 cells/cm². The cells were grown for 4, 6, or 7 days respectively in different brands of serum-free media. Cell proliferation was measured by adding alamarBlue[®] to each well, incubating for two hours, and then measuring fluorescence using a Wallac VICTOR2[™] MultiLabel Counter. The medium was not changed during the incubation period; the assay is a measure of a media's capacity to support log-phase growth over time. The higher the Relative Fluorescence Unit (RFU) value, the higher the rate of cell proliferation.

Supplier 1

Supplier 2

Supplier 3

ATCC [®] No.	Product Name	Components
PCS-300-010 [™]	Primary Bronchial/Tracheal Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-301-010 [™]	Primary Small Airway Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-300-030 [™]	Airway Epithelial Cell Basal Medium	485 mL
PCS-300-040 [™]	Bronchial Epithelial Cell Growth Kit	1 kit

ATCC Complete Airway Epithelial

Growth Medium

CARDIOVASCULAR CELLS

Each lot of ATCC Normal Human Primary Cardiovascular Cells is:

- Cryopreserved in the first or second passage to ensure the highest viability and plating efficiency
- Performance tested with ATCC Primary Cell Solutions media, kit supplements and reagents to guarantee optimum reliability
- Thoroughly tested for sample purity as part of the ATCC commitment to quality

The ATCC collection of primary cells includes cells isolated from different cardiovascular tissues

VASCULAR SMOOTH MUSCLE CELLS

- Aortic Smooth Muscle Cells, Normal, Human ATCC[®] <u>PCS-100-012</u>[™]
- Coronary Artery Smooth Muscle Cells, Normal, Human ATCC[®] <u>PCS-100-021</u>[™]
- Pulmonary Artery Smooth Muscle Cells, Normal, Human ATCC[®] <u>PCS-100-023</u>[™]

VASCULAR ENDOTHELIAL CELLS

- Umbilical Vein Endothelial Cells, Normal, Human ATCC[®] <u>PCS-100-010</u>[™]
- Umbilical Vein Endothelial Cells, Normal, Human, Pooled ATCC[®] <u>PCS-100-013</u>[™]
- Aortic Endothelial Cells, Normal, Human ATCC[®] <u>PCS-100-011</u>[™]
- Coronary Artery Endothelial Cells, Normal, Human ATCC[®] <u>PCS-100-020</u>™
- Pulmonary Artery Endothelial Cells, Normal, Human ATCC[®] <u>PCS-100-022</u>[™]
- Dermal Microvascular Endothelial Cells, Normal, Human, Neonatal ATCC[®] <u>PCS-110-010</u>[™]

These primary cells represent some of the most widely used models for in vitro research related to:

- Angiogenesis and normal microvascular growth
- Wound healing, vascular differentiation and tissue remodeling
- Arteriolosclerosis, hypertension and arterial disease

OPTIMIZED MEDIA SOLUTIONS

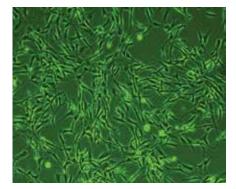
ATCC Vascular Cell Basal Medium provides an ideal cell culture environment for the propagation of cells under low-serum conditions (5% FBS or less), when supplemented with cell-specific growth kits.

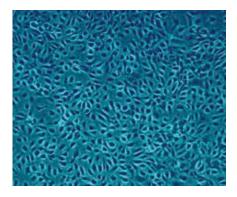
- Vascular Cell Basal Media ATCC[®] <u>PCS-100-030</u>[™]
- Vascular Smooth Muscle Cell Growth Kit ATCC[®] PCS-100-042[™]
- Endothelial Cell Growth Kit BBE ATCC[®] PCS-100-040[™]
- Endothelial Cell Growth Kit VEGF ATCC[®] PCS-100-041[™]
- Microvascular Endothelial Cell Growth Kit BBE ATCC[®] <u>PCS-110-040</u>[™]
- Microvascular Endothelial Cell Growth Kit VEGF ATCC[®] <u>PCS-110-041</u>[™]

Population Doublings of Primary Cardiovascular Cells in ATCC Complete Growth Media*

Cell Type and Medium	Average Doubling Time (hrs)
Umbilical Vein Endothelial Cells (BBE Growth Kit)	25.7
Umbilical Vein Endothelial Cells (VEGF Growth Kit)	22.7
Aortic Endothelial Cells (BBE Growth Kit)	28.6
Aortic Smooth Muscle Cells (SMC Growth Kit)	28.7

* All cells achieved ≥ 15 population doublings (approximately 4 passages) under these experimental conditions.







Growth of ATCC Primary Cell Solutions Cardiovascular Cells in Different Brands of Cell-Specific Low Serum or Classical Media

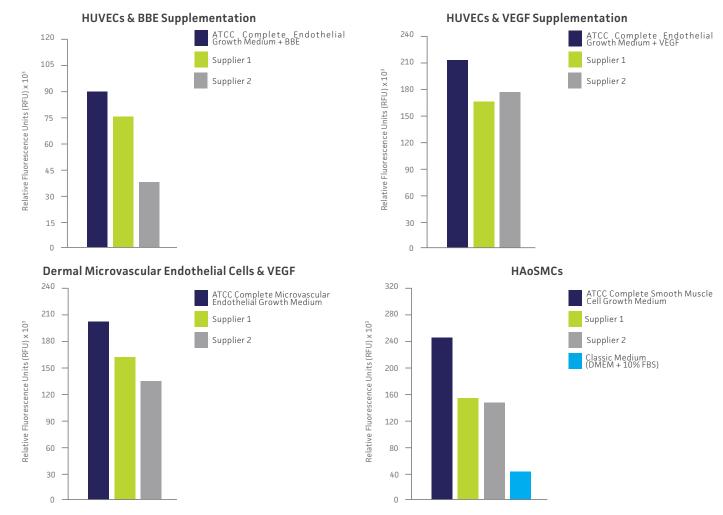
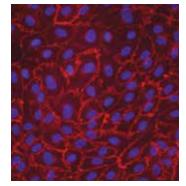


Figure 2: ATCC Primary Cell Solutions primary cells were taken from liquid nitrogen and cultures initiated. The cells were cultured for 3 to 4 days. The cells were then seeded in triplicate into a 24 well plate at 2,000 cells/cm² and grown for 4 days in different brands of low serum media or classical medium. Cell proliferation was measured by adding alamarBlue[®] to each well, incubating for two hours, and then measuring fluorescence using a Wallac VICTOR2[™] MultiLabel Counter. The medium was not changed during the incubation period; the assay is a measure of a media's capacity to support log-phase growth over time. The higher the Relative Fluorescence Unit (RFU) value, the higher the rate of cell proliferation.

Expression of Cell-Specific Markers

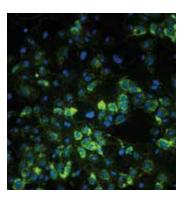


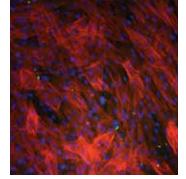
ATCC[®] <u>PCS-100-010</u>™

ATCC Primary Cell Solutions umbilical vein endothelial cells were stained for vascular endothelial cadherin (ve-cadherin) as a marker for endothelial cells. Red fluorescence indicates the expression of ve cadherin. DAPI was used as the nuclear stain.

ATCC[®] <u>PCS-100-011</u>™

ATCC Primary Cell Solutions aortic endothelial cells were dual stained for von Willebrand factor as a marker for endothelial cells and smooth muscle α -actin. Green fluorescence indicates the expression of von Willebrand factor and red fluorescence points to the expression of α -actin. The nuclei are blue with Hoechst stain. Expression of α -actin was not detectable, demonstrating the purity of the aortic endothelial cell preparation.





ATCC[®] <u>PCS-100-011</u>™

ATCC Primary Cell Solutions aortic endothelial cells were dual stained for von Willebrand factor as a marker for endothelial cells and smooth muscle a-actin. Green fluorescence indicates the expression of von Willebrand factor and red fluorescence points to the expression of α -actin. The nuclei are blue with Hoechst stain. Expression of α -actin was not detectable, demonstrating the purity of the aortic endothelial cell preparation.

ATCC [®] No.	Product Name	Components
<u>PCS-100-010</u> ™	Primary Umbilical Vein Endothelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-100-011 [™]	Primary Aortic Endothelial Cells; Normal, Human	\geq 5 x 10 ⁵ viable cells
<u>PCS-100-012</u> ™	Primary Aortic Smooth Muscle Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
<u>PCS-100-013</u> ™	Primary Umbilical Vein Endothelial Cells; Normal, Human, Pooled	\geq 5 x 10 ⁵ viable cells
<u>PCS-100-020</u> ™	Primary Coronary Artery Endothelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
<u>PCS-100-021</u> ™	Primary Coronary Artery Smooth Muscle Cells; Normal, Human	\geq 5 x 10 ⁵ viable cells
<u>PCS-100-022</u> ™	Primary Pulmonary Artery Endothelial Cells; Normal, Human	\geq 5 x 10 ⁵ viable cells
<u>PCS-100-023</u> ™	Primary Pulmonary Artery Smooth Muscle Cells; Normal, Human	\geq 5 x 10 ⁵ viable cells
<u>PCS-110-010</u> ™	Primary Dermal Microvascular Endothelial Cells; Normal, Human, Neonatal	≥ 5 x 10 ⁵ viable cells
<u>PCS-100-030</u> ™	Vascular Cell Basal Medium	475 mL
<u>PCS-100-040</u> ™	Endothelial Cell Growth Kit-BBE	1 kit
PCS-100-041 [™]	Endothelial Cell Growth Kit–VEGF	1 kit
<u>PCS-100-042</u> ™	Vascular Smooth Muscle Cell Growth Kit	1 kit
<u>PCS-110-040</u> ™	Microvascular Endothelial Cell Growth Kit–BBE	1 kit
<u>PCS-110-041</u> ™	Microvascular Endothelial Cell Growth Kit–VEGF	1 kit

CORNEAL CELLS

Each lot of ATCC Normal Human Primary Corneal Cells is:

- Cryopreserved in the second passage to ensure the highest viability and plating efficiency
- Performance tested together with ATCC Primary Cell Solutions[®] media, kit supplements and reagents to guarantee optimum reliability
- Thoroughly tested for sample purity as part of the ATCC commitment to quality

CORNEAL EPITHELIAL CELLS

Corneal Epithelial, Normal, Human - ATCC[®] PCS-700-010[™]

These cells represent some of the most widely used models for reserach related to:

Gene regulation

Page 8

Tissue development

OPTIMIZED MEDIA SOLUTIONS

Primary corneal epithelial cells are effectively supported by the cell-specific ATCC Primary Cell Solutions system consisting of Corneal Epithelial Cell Basal Medium supplemented with the Corneal Epithelial Cell Growth Kit. This unique formulation is designed to produce cultures with superior growth and proliferation and normal morphology.

Use of this complete system removes the need for additional components such as feeder layers, extracellular matrix proteins or other substrates.

- Corneal Epithelial Cell Basal Media ATCC[®] PCS-700-030[™]
- Corneal Epithelial Cell Growth Kit ATCC[®] PCS-700-040[™]

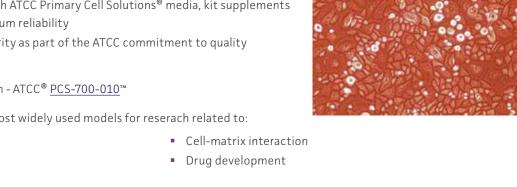
Relative Flourescence Units (RFU) x 10^3

225 200

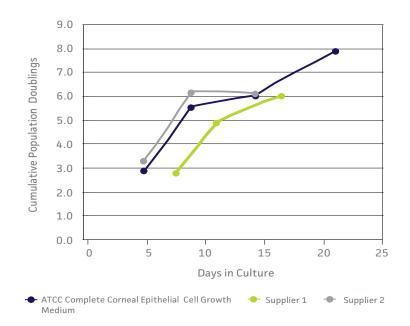
Growth of Primary Human Corneal Epithelial Cells in Different Brands of Serum-Free Media

0 Day 4 Day 6 ATCC Complete Supplier 1 Supplier 2 Corneal Epithelial Cell Basal Medium Figure 3: ATCC Primary Cell Solutions corneal epithelial cells were taken from liquid nitrogen and cultures initiated. The cells were cultured for 3 to 4 days. The cells were then seeded in triplicate into a 24-well plate at 1,500 cells/cm², 600 cells/cm², or 400 cells/cm², and grown for 4, 6, or 7 days respectively in different brands of serum-free media. Cell proliferation was measured by removing the medium and adding 0.05% Crystal Violet stain solution and incubating for 30 minutes at room temperature. The plates were rinsed and then allowed to air-dry. The dried stain was then resolubilized using alcohol. Absorbance at 590 nm was measured using a Wallac VICTOR2™ MultiLabel Counter. The medium was not changed during the incubation period; the assay is a measure of a media's capacity to support log-phase growth over time. The higher the absorbance value, the higher the rate of cell proliferation.



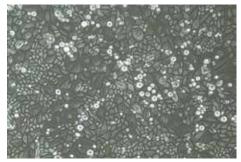


Population Doubling: Corneal Epithelial Cells in Different Brands of Serum-Free Media

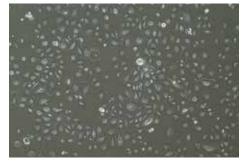


This experiment was conducted while various lots of ATCC Primary Cell Solutions corneal epithelial cells were undergoing QC testing. When grown in ATCC Complete Growth Medium, primary corneal epithelial cells maintained normal morphology and growth characteristics through four passages after thaw. The morphology of cells grown in Supplier 1 Medium was unsatisfactory after two passages and cell growth drastically slowed; the cells were discarded in passage 3. Cells grown in Supplier 2 Medium appeared to stop dividing after passage 2. (See cell photos and population doubling times on page 3.)

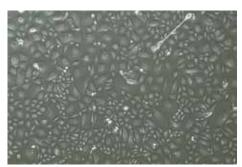
Comparison of ATCC Primary Cell Solutions Corneal Epithelial Cell Morphology in Different Brands of Media



ATCC Complete Corneal Epithelial Cell Growth Medium (passage 2, day 4)



Supplier 1 Medium (passage 2, day 2)



Supplier 2 Medium (passage 2, day 4)

Population Doubling Times by Passage Number

Medium	1	2	3	4	
ATCC Complete Corneal Epithelial Cell Growth Medium	34.8 h	24.8 h	73.5 h	102 h	
Supplier 1	54.6 h	46.6 h	104 h	N/A	
Supplier 2	31.4 h	30.6 h	N/A	N/A	

ATCC [®] No.	Product Name	Components
<u>PCS-700-010</u> ™	Primary Corneal Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-700-030 [™]	Corneal Epithelial Cell Basal Medium	485 mL
<u>PCS-700-040</u> ™	Corneal Epithelial Cell Growth Kit	1 kit

MAMMARY CELLS

Each lot of ATCC Normal Human Primary Mammary Epithelial Cells is:

- Cryopreserved at early passage (P2) to ensure the greatest purity, high viability, and good plating efficiency.
- Performance tested with ATCC Primary Cell Solutions Media, kit supplements, and reagents to guarantee optimum reliability.
- Thoroughly tested for sample purity as part of the ATCC commitment to quality.

The ATCC collection of primary cells includes cells isolated from mammary tissue.

MAMMARY EPITHELIAL CELLS

Mammary Epithelial Cells, Normal, Human - ATCC[®] <u>PCS-600-010</u>[™]

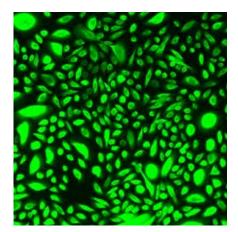
These are among the most widely used models for in vitro research related to:

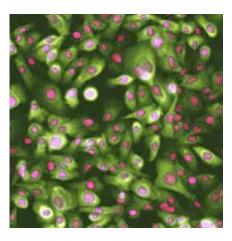
- Normal breast development
- Breast cancer oncogenesis and metastasis
- drug screening
- 3D modeling

OPTIMIZED MEDIA SOLUTIONS

ATCC basal media are intended to serve as the foundation of a culture system that is tailored to the needs of a particular cell by the addition of an ATCC growth kit. The Mammary Epithelial Cell Growth Kit contains aliquots of rH-Insulin, L-Glutamine, Epinephrine, Apo-Transferrin, rH-TGFα, Extract P, and Hydrocortisone Hemisuccinate. These are added to Mammary Epithelial Basal media to provide optimized conditions to support epithelial cells from the human mammary gland. This unique formulation is designed to produce cultures with functional expression of relevant biomarkers, and normal growth, proliferation, and morphology.

- Mammary Epithelial Cell Basal Medium ATCC[®] <u>PCS-600-030</u>[™]
- Mammary Epithelial Cell Growth Kit ATCC[®] <u>PCS-600-040</u>[™]





MESENCHYMAL STEM CELLS

Each lot of ATCC Mesenchymal Stem Cells is:

- Cryopreserved in the first or second passage to ensure the highest viability and plating efficiency
- Performance tested together with ATCC Primary Cell Solutions[®] media, kit supplements and reagents to guarantee optimum reliability
- Thoroughly tested for sample purity as part of the ATCC commitment to quality
- Verified for surface antigen expression, including a total of 10 markers: Positive (≥95%) for CD29, CD44, CD73, CD90, CD105, and CD166; and, negative (2%) for CD14, CD31, CD34, CD45
- Confirmed to retain multi-lineage differentiation into osteoblasts, adipocytes, and chondrocytes using optimized differentiation kits and protocols

Mesenchymal stem cells are capable of replicating as undifferentiated cells or differentiating down multiple pathways to form bone, cartilage, fat, muscle, tendon/ligament tissues or marrow stroma.

The ATCC collection of Mesenchymal Stem Cells includes:

ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

- Adipose-derived Mesenchymal Stem Cells, Normal, Human ATCC[®] PCS-500-011[™]
- Primary Subcutaneous Pre-adipocytes, Normal, Human ATCC[®] <u>PCS-210-010</u>[™]

BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Bone Marrow-derived Mesenchymal Stem Cells, Normal, Human - ATCC[®] PCS-500-012[™]

UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

■ Umbilical Cord-derived Mesenchymal Stem Cells, Normal, Human - ATCC[®] <u>PCS-500-010</u>™

ATCC MSCs are good in vitro models for research related to:

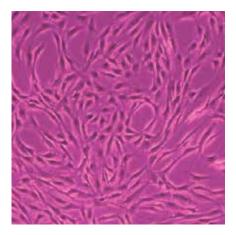
- Adult stem cell differentiation
- Tissue engineering, cell therapy, and regenerative medicine
- Generation of induced pluripotent stem cell lines (1,2)

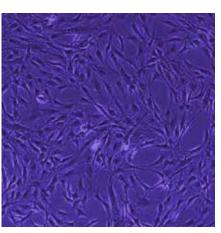
¹Schäffler, A and Büchler, C. Concise Review: Adipose Tissue-Derived Stromal Cells—Basic and Clinical Implications for Novel Cell-Based Therapies. Stem Cells 2007; 25: 818-827. ²Sun, N et al. Feeder-Free Derivation of Induced Pluripotent Stem Cells from Adult Human Adipose Stem Cells. PNAS 2009; 106: 15720-15725



Phenotypic Profile of Mesenchymal Stem Cells

ATCC[®] Primary Cell Solutions[®] Adipose-Derived Mesenchymal Stem Cells were taken from liquid nitrogen and cultures initiated. A sample for analysis by flow cytometry was taken when the culture was initiated and then after 48-hours of growth. The cells must test positive for CD29, CD44, CD73, CD90, CD105, and CD166 (greater than 95% of the cell population expresses these markers by flow cytometry). The cells must test negative for CD14, CD31, CD34, and CD45 (less than 2% of cell population expresses these markers by flow cytometry).





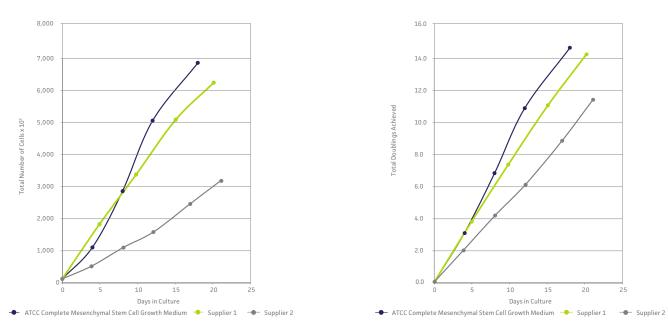
OPTIMIZED MEDIA SOLUTIONS

Primary mesenchymal stem cells are effectively supported by the cell-specific ATCC Primary Cell Solutions system consisting of Mesenchymal Stem Cell Basal Medium supplemented with the Mesenchymal Stem Cell Growth Kit–Low serum. This unique formulation is designed to produce cultures with functional expression of relevant biomarkers, superior growth and proliferation, normal morphology, multipotent lineage-specific differentiation.

Use of this complete system removes the need for additional components such as feeder layers, extracellular matrix proteins or other substrates.

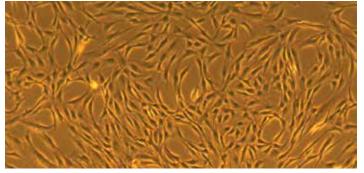
- Mesenchymal Stem Cell Basal Media ATCC[®] <u>PCS-500-030</u>[™]
- Mesenchymal Stem Cell Growth Kit Low Serum ATCC[®] <u>PCS-500-040</u>[™]
- Mesenchymal Stem Cell Growth Kit for Bone Marrow MSCs Low serum ATCC[®] <u>PCS-500-041</u>[™]

Growth Rate Comparison*: Adipose-Derived Mesenchymal Stem Cells Cultured in Different Brands of Media

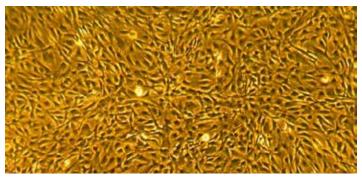


*This experiment was conducted while various lots of ATCC[®] Primary Cell Solutions[®] Adipose-Derived Mesenchymal Stem Cells were undergoing QC testing. When the QC-specification for population doublings was achieved (≥15) the experiment was concluded; cells grown in Supplier 2 medium did not achieve 15 population doublings.

Normal Morphology



ATCC Complete Mesenchymal Stem Cell Growth Medium maintains adipose-derived mesenchymal stem cells in an undifferentiated state with normal morphology. Passage 3 cells, day 4 (100X magnification).



ATCC Complete Mesenchymal Stem Cell Growth Medium maintains umbilical cord-derived mesenchymal stem cells in an undifferentiated state with normal morphology. Passage 5 cells, day 7 (100X magnification).

DIFFERENTIATION MADE SIMPLE

ATCC Differentiation Tools allow for fast, easy and affordable manipulation of ATCC Normal Human Mesenchymal Stem Cells into three mesenchymal lineages in vitro, including:

- Adipocyte Differentiation Toolkit for Adipose Derived MSCs and Preadipocytes ATCC[®] PCS-500-050[™]
- Chondrocyte Differentiation Tool ATCC[®] <u>PCS-500-051</u>™
- Osteocyte Differentiation Tool ATCC[®] <u>PCS-500-052</u>™

Each tool is formulated and optimized for use with ATCC Adipose-Derived Mesenchymal Stem Cells and ATCC Umbilical Cord-Derived Mesenchymal Stem Cells, to induce reliable differentiation for rapid results.

In fact, it's so easy to use that ATCC Normal Human Mesenchymal Stem Cells can be differentiated into adipocytes, chondrocytes or osteocytes in just 4 basic steps using ATCC Differentiation Tools:

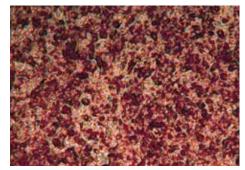
Step 1: Culture ATCC Normal Human Mesenchymal Stem Cells and prepare the cells for differentiation.

Step 2: Initiate differentiation; renew medium at regular intervals.

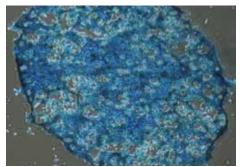
Step 3: Renew the differentiation medium or add a maintenance medium to support differentiation.

Step 4: Analyze fully-differentiated, mature cells. Confirm differentiation by staining.

THE RIGHT TOOLS WILL PRODUCE THE RESULTS YOU WANT



ATCC Adipose-Derived Mesenchymal Stem Cells were expanded in Complete Growth Medium and then induced to differentiate into adipocytes using the Adipocyte Differentiation Toolkit. The cells are stained with Oil Red O. Passage 3 cells, day 18 following differentiation (100X magnification).



ATCC Adipose-Derived Mesenchymal Stem Cells were expanded in Complete Growth Medium and then induced to differentiate to chondrocytes using the Chondrocyte Differentiation Tool. Passage 3 cells, day 21 following differentiation (100X magnification), stained with Alcian Blue.



ATCC Adipose-Derived Mesenchymal Stem Cells were expanded in Complete Growth Medium and then induced to differentiate to osteocytes using the Osteocyte Differentiation Tool. Passage 5 cells, day 21 following differentiation (40X magnification), stained with Alizarin Red S.

ATCC [®] No.	Product Name	Components
<u>PCS-210-010</u> ™	Subcutaneous Pre-Adipocytes; Normal, Human	\geq 1 x 10 ⁶ viable cells
PCS-500-010 [™]	Umbilical Cord-Derived Mesenchymal Stem Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
<u>PCS-500-011</u> ™	Adipose-Derived Mesenchymal Stem Cells; Normal, Human	≥ 1 x 10 ⁶ viable cells
PCS-500-012 [™]	Bone Marrow-Derived Mesenchymal Stem Cells; Normal, Human	\geq 1 x 10 ⁶ viable cells
<u>PCS-500-030</u> ™	Mesenchymal Stem Cell Basal Medium for Adipose, Umbilical and Bone Marrow derived MSCs	485 mL
<u>PCS-500-040</u> ™	Mesenchymal Stem Cell Growth Kit for Adipose and Umbilical-derived MSCs – Low serum	1 kit
<u>PCS-500-041</u> ™	Mesenchymal Stem Cell Growth Kit for Bone Marrow MSCs – Low serum	1 kit
PCS-500-050™	Adipocyte Differentiation Toolkit for Adipose Derived MSCs and Preadipocytes	1 kit
<u>PCS-500-051</u> ™	Chondrocyte Differentiation Tool	1 kit
<u>PCS-500-052</u> ™	Osteocyte Differentiation Tool	1 kit

PROSTATE CELLS

Each lot of ATCC Normal Human Primary Prostate Cells is:

- Cryopreserved in the second passage to ensure the highest viability and plating efficiency
- Performance tested together with ATCC Primary Cell Solutions[®] media, kit supplements ,and reagents to guarantee optimum reliability
- Thoroughly tested for sample purity as part of the ATCC commitment to quality.
- The ATCC collection of primary cells includes cells isolated from prostate tissue.
- Prostate Epithelial Cells, Normal, Human ATCC[®] <u>PCS-440-010</u>[™]

These prostate epithelial cells represents some of the most widely used models for in vitro research related to:

Hormonal regulation

- Prostate secretory regulation
- Prostate Cancer

OPTIMIZED MEDIA SOLUTIONS

Primary prostate epithelial cells are effectively supported by the cell-specific ATCC Primary Cell Solutions system consisting of Prostate Epithelial Cell Basal Medium supplemented with the Prostate Epithelial Cell Growth Kit. This unique formulation is designed to produce cultures with functional expression of relevant biomarkers, superior growth and proliferation, and normal morphology.

Use of this complete system removes the need for additional components such as feeder layers, extracellular matrix proteins or other substrates.

- Prostate Epithelial Cell Basal Media ATCC[®] <u>PCS-440-030</u>[™]
- Prostate Epithelial Cell Growth Kit ATCC[®] PCS-440-040[™]

Growth of ATCC Primary Cell Solutions Primary Human Prostate Epithelial Cells in Different Brands of Serum-Free Media

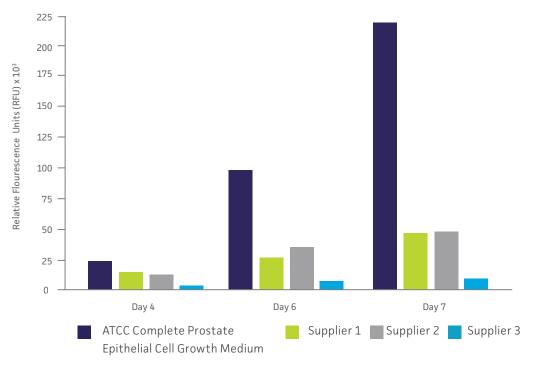
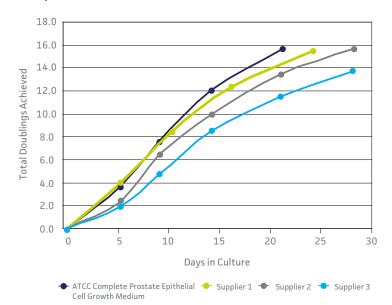


Figure 4: ATCC Primary Cell Solutions prostate epithelial cells were taken from liquid nitrogen and cultures initiated. The cells were cultured for 3 to 4 days. The cells were then seeded in triplicate into a 24-well plate at 1,000 cells/cm², 600 cells/cm², or 400 cells/cm², and grown for 4, 6, or 7 days respectively in different brands of serum-free media. Cell proliferation was measured by removing the medium and adding 0.05% Crystal Violet stain solution and incubated. The plates were rinsed and then allowed to air-dry. The dried stain was then resolubilized using alcohol. Absorbance at 590 nm was measured using a Wallac VICTOR2[™] MultiLabel Counter. The medium was not changed during the incubation period; the assay is a measure of a media's capacity to support log-phase growth over time. The higher the absorbance value, the faster the rate of cell proliferation.



Growth Rate Comparison*: Prostate Epithelial Cells Cultured in Different Brands of Serum-Free Media



Medium	Number of Doublings	Days in Culture	Average Doubling Time (hrs)
ATCC Complete Prostate Epithelial Cell Growth Medium	15.7	21	32.9
Supplier 1	15.5	24	38.8
Supplier 2	15.6	28	47.7
Supplier 3	13.7	28	52.5

ATCC [®] No.	Product Name	Components
<u>PCS-440-010</u> ™	Primary Prostate Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
<u>PCS-440-030</u> ™	Prostate Epithelial Cell Basal Medium	485 mL
<u>PCS-440-040</u> ™	Prostate Epithelial Cell Growth Kit	1 kit

-0

RENAL CELLS

Each lot of ATCC Normal Human Primary Renal Cells is:

- Cryopreserved in the first or second passage to ensure the highest viability and plating efficiency.
- Performance tested with ATCC Primary Cell Solutions media, kit supplements and reagents to guarantee optimum reliability.
- Thoroughly tested for sample purity as part of the ATCC commitment to quality.

The ATCC collection of primary cells includes cells isolated from different regions of the kidney.

RENAL EPITHELIAL CELLS

- Renal Proximal Tubule Epithelial Cells, Normal, Human ATCC[®] PCS-400-010[™]
- Renal Cortical Epithelial Cells, Normal, Human ATCC[®] <u>PCS-400-011</u>[™]
- Renal Mixed Epithelial Cells, Normal, Human ATCC[®] <u>PCS-400-012</u>[™]

Primary, normal renal cells are the ideal model for research related to:

- Hypertension and diabetes
- Renal fibrosis, inflammation, and cancer.
- Drug screening, development and toxicity assessment.

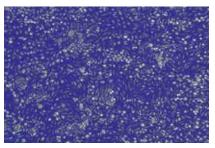
OPTIMIZED MEDIA SOLUTIONS

Primary renal epithelial cells are effectively supported by the complete ATCC Primary Cell Solutions cell system consisting of Renal Epithelial Cell Basal Medium supplemented with the Renal Epithelial Cell Growth Kit. This unique formulation is designed to produce cultures with functional expression of relevant biomarkers, high growth rate and normal morphology.

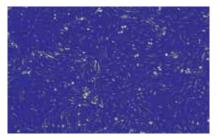
Use of this complete system eliminates the need for additional components such as feeder layers, extracellular matrix proteins or other substrates to enhance attachment and proliferation. Cells grown in complete Renal Epithelial Cell Growth Medium are clear, highly refractile, and have a more compact cuboidal morphology compared to cells grown in other brands of renal epithelial media. More compact cells means more cells per flask.

- Renal Epithelial Cell Basal Media ATCC[®] <u>PCS-400-030</u>™
- Renal Epithelial Cell Growth Kit ATCC[®] <u>PCS-400-040</u>[™]

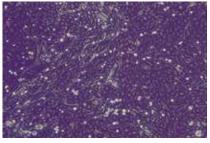
Comparison of ATCC Primary Cell Solutions Renal Proximal Tubule Epithelial Cell Morphology and Cell Density in Different Complete Renal Epithelial Cell Growth Media*



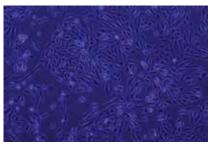
ATCC Complete Renal Epithelial Cell Growth Medium



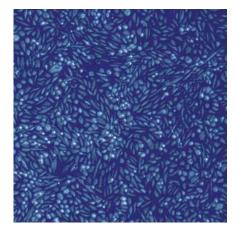
Supplier 2 Medium *All cells were imaged at passage 4.



Supplier 1 Medium



Supplier 3 Medium



Renal Proximal Tubal Epithelial Cells in Different Brands of Low Media: Levels of y-glutamyl Transferase Activity

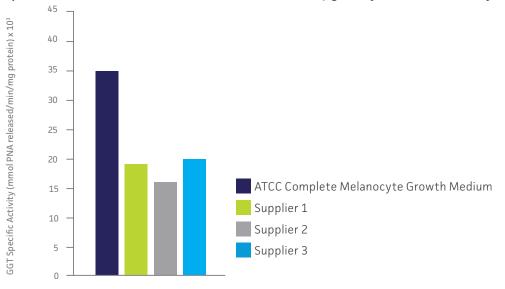
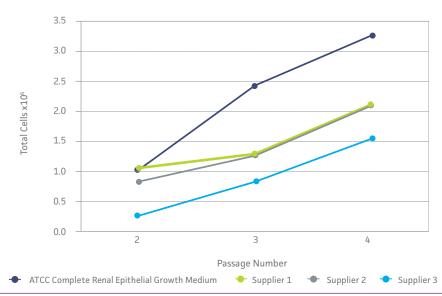


Figure 5: ATCC Primary Cell Solutions Renal Proximal Tubule Epithelial Cells were taken from liquid nitrogen and cultures initiated. The cells were grown for 4 days and then seeded in quintuplicate into a 24-well plate at 1,000 cells/cm2 and grown in different brands of low serum medium for 13 days with periodic changes of media. 500 µL of assay buffer (150 mM Tris-HCl [pH 8.0], 187.5 mM NaCl, 0.2% Triton X-100) was added to each well and the plates were agitated on an orbital shaker for at least 5 minutes to solubilize the cells. The contents of 5 wells per medium were combined and total protein measured using the Bio-Rad Protein Assay (Bradford method). 400 µL of assay buffer containing solubilized cell material, 400 µL of 100 mM Gly-Gly (pH 8.0) reagent, and 200 µL of 12.5 mM L-gamma-glutamyl-p-nitro-anilide (PNA) were combined and incubated at 37°C for 30 min. Absorbance at 410 nm was measured using a Wallac VICTOR2[™] MultiLabel Counter. The activity of the samples was calculated against a standard curve of the reaction product, p-nitroaniline.

Comparison of Total Renal Proximal Tubule Cell Number Over Time



ATCC [®] No.	Product Name	Components
PCS-400-010	Primary Renal Proximal Tubule Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-400-011	Primary Renal Cortical Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-400-012	Primary Renal Mixed Epithelial Cells; Normal, Human	≥ 5 x 10 ⁵ viable cells
PCS-400-030	Renal Epithelial Cell Basal Medium	485 mL
PCS-400-040	Renal Epithelial Cell Growth Kit	1 kit

Table 1: ATCC Primary Cells and Needed Reagents

All of these ATCC primary cells products are human species and require the following reagents: Phenol Red (ATCC PCS-999-001); D-PBS (ATCC 30-

Product Name	Applications	Number of viable cells-post thaw	
	Applications	Liidw	Treezing
Endothelial Cells	Dissiple size and electropical investigations, such as macromolecule transport blood	~5.0 v 10 ⁵	1
Umbilical Vein Endothelial Cells; Normal, Human (PCS-100-010)	Physiological and pharmacological investigations, such as macromolecule transport, blood coagulation, angiogenesis, and fibrinolysis	$\ge 5.0 \times 10^5$ $\ge 5.0 \times 10^5$	
Umbilical Vein Endothelial Cells; Normal, Human, Pooled (PCS-100-013) Aortic Endothelial Cells; Normal, Human (PCS-100-011)		≥5.0 x 10 ⁵ ≥5.0 x 10 ⁵	
	Studies of vascular diseases such as thrombosis, atherosclerosis, metabolism, and hypertension, stent-graft compatibility testing, and membrane conductance models	≥5.0 x 10 ⁵ ≥5.0 x 10 ⁵	
Coronary Artery Endothelial Cells; Normal, Human (PCS-100-020)		≥5.0 x 10 ⁵ ≥5.0 x 10 ⁵	
Pulmonary Artery Endothelial Cells; Normal, Human (PCS-100-022)	Ct. 11 C. Second Street Second Street Second Street		
Dermal Microvascular Endothelial Cells; Normal, Human, Neonatal (PCS-110-010)	Studies of microvascular functions and cutaneous inflammation	≥5.0 x 10 ⁵	3
Smooth Muscle Cells			
Aortic Smooth Muscle Cells; Normal, Human (PCS-100-012)	Studies of vascular diseases, such as thrombosis and atherosclerosis	≥5.0 x 10 ⁵	
Coronary Artery Smooth Muscle Cells; Normal, Human (PCS-100-021)		≥5.0 x 10 ⁵	
Pulmonary Artery Smooth Muscle Cells; Normal, Human (PCS-100-023)		≥5.0 x 10 ⁵	
Lung Smooth Muscle Cells; Normal, Human (PCS-130-010)		≥5.0 x 10 ⁵	2
Bronchial/Tracheal Smooth Muscle Cells; Normal, Human (PCS-130-011)		≥5.0 x 10 ⁵	2
Bladder Smooth Muscle Cells; Normal, Human (PCS-420-012)		≥5.0 x 10 ⁵	2
Uterine Smooth Muscle Cells; Normal, Human (PCS-460-010)		≥5.0 x 10 ⁵	2
Epithelial Cells			
Small Airway Epithelial Cells; Normal, Human (PCS-301-010)	Asthma, airway inflammation, and wound healing, pulmonary fibrosis, COPD, cancer,	≥5.0 x 10 ⁵	1
Small Airway Epitheliai Cells; Normal, Human (PCS-301-010) Bronchial/Tracheal Epithelial Cells; Normal, Human (PCS-300-010)	Astima, airway inflammation, and wound nearing, purmonary fibrosis, COPD, cancer, toxicology, intracellular pH regulations, IL-1b effect to stimulate airway epithelial cell growth, and ICAM-1 expression		
Lobar Bronchial Epithelial Cells; Normal, Human (PCS-300-015)	Asthma, airway inflammation, and wound healing, pulmonary fibrosis, COPD, cancer, toxicology,	≥5.0 x 10 ⁵	1
	intracellular pH regulations, IL-1b effect to stimulate airway epithelial cell growth, and ICAM-1 expression	20.0 A 20	
Renal Proximal Tubule Epithelial Cells; Normal, Human (PCS-400-010)	In vitro studies of osmoregualtion and excretion, renal fibrosis, inflammation, as well as	≥5.0 x 10 ⁵	2
Renal Cortical Epithelial Cells; Normal, Human (PCS-400-011)	applications in drug screening/development, eg, hypertension, diabetes, oncology,	≥5.0 x 10 ⁵	1
Renal Mixed Epithelial Cells; Normal, Human (PCS-400-012)	- autoimmune disease, and toxicology screening	≥5.0 x 10 ⁵	
Bladder Epithelial Cells (A/T/N); Normal, Human (PCS-420-010)	Incontinence and reconstruction studies or development of a potential diagnostic method for the early detection of bladder cancer cells	≥5.0 × 10 ⁵	
Prostate Epithelial Cells; Normal, Human (PCS-440-010)	Hormonal regulation of the prostate, the secretory function of prostate cells, and prostate cancer cancer	≥5.0 x 10 ⁵	2
Vaginal Epithelial Cells; Normal, Human (PCS-480-010)	Cancer studies, microbiological organism to cell interaction, toxicity studies	≥5.0 x 10 ⁵	3
Cervical Epithelial Cells; Normal, Human (PCS-480-011)	Pathophysiology of cervical polyps, HPV, and cervical cancer	≥5.0 x 10 ⁵	3
Mammary Epithelial Cells; Normal, Human (PCS-600-010)	Breast cancer development, and 3-D culture and carcinogen screening	≥5.0 x 10 ⁵	3
Corneal Epithelial Cells; Normal, Human (PCS-700-010)	Cell de-differentiation, toxicology testing, and drug development	≥5.0 x 10 ⁵	2
Fibroblasts			
Dermal Fibroblasts; Normal, Human Neonatal (PCS-201-010)	Wound healing studies, tissue engineering and regeneration applications, as well as induction of pluripotent stem (iPSCs)	≥5.0 x 10 ⁵	1
Dermal Fibroblasts; Normal, Human Adult (PCS-201-012)	Wound healing studies, tissue engineering and regeneration applications, as well as induction of pluripotent stem (IPSCs)	≥5.0 x 10 ⁵	1
Lung Fibroblasts; Normal, Human (PCS-201-013)	Lung disorders and infections, lung reconstruction studies, and advancement of cancer research	>5.0 x 10 ⁵	2
Gingival Fibroblasts; Normal, Human (PCS-201-013)	Regenerative medicine studies, alternate source of MSCs	≥5.0 x 10 ≥5.0 x 10 ⁵	
		≥5.0 x 10 ⁵ ≥5.0 x 10 ⁵	
Bladder Fibroblasts; Normal, Human (PCS-420-013)	Detection of bladder cancer cells, reconstruction studies, and advancement of cancer research Research related to female reproductive biology, drug testing, and appendence		2
Uterine Fibroblast; Normal, Human (PCS-460-010)	Research related to female reproductive biology, drug testing, and oncology	≥5.0 x 10 ⁵	2
Keratinocytes			
Epidermal Keratinocytes; Normal, Human, Neonatal Foreskin (PCS-200-010)	Studies of growth factor activity, wound healing, toxicity/irritancy studies, and use as target		
Epidermal Keratinocytes; Normal, Human, Adult (PCS-200-011)	cells for derivation of induced pluripotent stem cells	≥5.0 x 10 ⁵	
Gingival Keratinocytes; Normal, Human (PCS-200-014)	Antibiotic treatment, dental implants, and many other applications for oral biology research	≥5.0 x 10 ⁵	2
Melanocytes			
Epidermal Melanocytes; Normal, Human, Neonatal Foreskin (PCS-200-012)	Wound healing, testing models for toxicity/irritancy studies, melanoma, dermal response to	≥5.0 x 10 ⁵	2
Epidermal Melanocytes; Normal, Human, Adult (PCS-200-013)	UV radiation, psoriasis and other skin diseases, and cosmetic research	≥5.0 x 10 ⁵	
Mesenchymal Stem Cells			
Umbilical Cord-Derived Mesenchymal Stem Cells; Normal, Human (PCS-500-010)	Adult stem cell differentiation research, induced pluripotent stem cell lines, tissue engineering, cell therapy, and regenerative medicine	≥5.0 x 10 ⁵	2
Adipose-Derived Mesenchymal Stem Cells; Normal, Human (PCS-500-011)		≥1.0 x 10 ⁶	2
Bone Marrow-Derived Mesenchymal Stem Cells; Normal, Human (PCS-500-012)	Useful as an in vitro model for the study of multipotent stem cell biology, differentiation, and regenerative medicine and tissue engineering	≥1.0 x 10 ⁶	2
Primary Subcutaneous Preadipocytes; Normal, Human (PCS-210-010)	Differentiation research, tissue engineering, cell therapy, and regenerative medicine	≥1.0 x 10 ⁶	2
Skeletal Muscle Cells			
Skeletal Muscle Cells; Normal, Human (PCS-950-010)	Ideal culture model for the study of muscle cell biology, diabetes, insulin receptor studies, muscle cell metabolism, muscle tissue repair, and myotube development	≥5.0 x 10 ⁵	3

2200); Trypsin-EDTA for Primary Cells (ATCC PCS-999-003); Trypsin Neutralizing Solution (ATCC PCS-999-004).

thaw to achieve	Basal media	Growth kit	Additional Reagents
≥15 PDL	Vascular Cell Basal Medium (PCS-100-030)	Endothelial Cell Growth Kit-BBE (PCS-100-040) or Endothelial Cell	Not applicable
≥15 PDL		Growth Kit-VEGF (<u>PCS-100-041</u>)	Not applicable
≥15 PDL			
≥15 PDL	-		
≥15 PDL	-		
≥15 PDL	Vascular Cell Basal Medium (<u>PCS-100-030</u>)	Microvascular Endothelial Cell Growth Kit-BBE (<u>PCS-110-040</u>) or Microvascular Endothelial Cell Growth Kit-VEGF (<u>PCS-110-041</u>)	_
≥15 PDL	Vascular Cell Basal Medium (<u>PCS-100-030</u>)	Vascular Smooth Muscle Cell Growth Kit (<u>PCS-100-042</u>)	Notapplicable
≥15 PDL			
≥15 PDL	-		
>15 PDL >15 PDL			
>15 PDL			
>15 PDL	-		
≥15 PDL	Airway Epithelial Cell Basal Medium - (<u>PCS-300-030</u>)	Small Airway Epithelial Cell Growth Kit (PCS-301-040) or Bronchial Epithelial Cell Growth kit (<u>PCS-300-040</u>)	Not applicable
≥15 PDL			_
>10 PDL	Airway Epithelial Cell Basal Medium (<u>PCS-300-030</u>)	Bronchial Epithelial Cell Growth Kit (<u>PCS-300-040</u>)	
≥15 PDL	Renal Epithelial Cell Basal Medium	Renal Epithelial Cell Growth Kit (PCS-400-040)	-
≥15 PDL	(<u>PCS-400-030</u>)		
≥15 PDL	-		
>15 PDL	Bladder Epithelial Basal Medium (<u>PCS-420-032</u>)	Bladder Epithelial Growth Kit (<u>PCS-420-042</u>)	_
≥15 PDL	Prostate Epithelial Cell Basal Medium (<u>PCS-440-030</u>)	Prostate Epithelial Cell Growth Kit (<u>PCS-440-040</u>)	_
>10 PDL	Vaginal Epithelial Basal Medium (<u>PCS-480-030</u>)	Vaginal Epithelial Growth Kit (<u>PCS-480-040</u>)	
>10 PDL	Cervical Epithelial Cell Basal Medium (<u>PCS-480-032</u>)	Cervical Epithelial Cell Growth Kit (<u>PCS-480-042</u>)	_
>15 PDL	Mammary Epithelial Cell Basal Medium (<u>PCS-600-030</u>)	Mammary Epithelial Cell Growth Kit (<u>PCS-600-040</u>)	_
3 passages	Corneal Epithelial Cell Basal Medium (<u>PCS-700-030</u>)	Corneal Epithelial Cell Growth Kit (<u>PCS-700-040</u>)	
≥10 PDL in	Fibroblast Basal Medium (PCS-201-030)	Fibroblast Growth Kit–Serum-Free (PCS-201-040) or Fibroblast	0.1% Gelatin Solution (PCS-999-027) only for use
serum-free medium		Growth Kit–Low Serum (<u>PCS-201-041</u>)	with Mitomicin C treated Dermal Fibroblasts
≥10 PDL in	-		
serum-free medium			
>15 PDL			Notapplicable
>15 PDL			
>15 PDL	-		
>15 PDL			
≥15 PDL	Dermal Cell Basal Medium (PCS-200-030)	Keratinocyte Growth Kit (PCS-200-040)	Not applicable
≥15 PDL			
>15 PDL	•		
≥15 PDL	Dermal Cell Basal Medium (PCS-200-030)	Melanocyte Growth Kit (PCS-200-041)	Not applicable
≥15 PDL			
≥10 PDL	Mesenchymal Stem Cell Basal Medium (PCS-500-030)	Mesenchymal Stem Cell Growth Kit-Low Serum (<u>PCS-500-040</u>)	Adipocyte Differentiation Tool (<u>PCS-500-050</u>)
≥10 PDL			Chondrocyte Differentiation Tool (<u>PCS-500-051</u>)
≥10 PDL		Mesenchymal Stem Cell Growth Kit for Bone Marrow MSCs (PCS-500-041)	Osteocyte Differentiation Tool (<u>PCS-500-052</u>) Adipocyte Differentiation Toolkit for BM-MSCs (PCS-500-053)
≥15 PDL	Fibroblast Basal Medium (PCS-201-030)	(PCS-201-041)	Adipocyte Differentiation Tool (PCS-500-050)
>10 PDL	Mesenchymal Stem Cell Basal Medium (PCS-500-030)	Skeletal Muscle Growth Kit (<u>PCS-950-040</u>)	Skeletal Muscle Differentiation Tool (<u>PCS-950-050</u>)



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