ATCC

Product Sheet

Primary Dermal Microvascular Endothelial Cells; Normal, Human, Neonatal (ATCC® PCS-110-010™)

Please read this FIRST

Storage Temp.
-130°C or below

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Primary Dermal Microvascular Endothelial Cells; Normal, Human, Neonatal (ATCC® PCS-110-010™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

ATCC® Normal Human Primary Dermal Microvascular Endothelial Cells from Neonatal Foreskin (HDMVECn), when grown in Vascular Cell Basal Media supplemented with Microvascular Endothelial Cell Growth Kit components, provide an ideal cell system to propagate microvascular endothelial cells in low serum conditions with or without the addition of human recombinant VEGF. The cells are cryopreserved in the third (or fourth) passage to ensure the greatest purity of the cell population, coupled with high viability and plating efficiency. ATCC® Primary Cell Solutions™ cells, media, supplements and reagents are quality-tested together to guarantee optimum performance and reliability.

Components: One vial Primary Dermal Microvascular Endothelial Cells; Normal, Human, Neonatal (ATCC® PCS-110-010™) containing a minimum of 5 x 10^5 viable cells (provided).

Also Required:

A. One bottle of Vascular Cell Basal Medium (ATCC PCS-100-030) plus one Microvascular Endothelial Cell Growth Kit of either:
   1. Microvascular Endothelial Cell Growth Kit-BBE (ATCC PCS-110-040) containing each of the following growth supplements: Bovine Brain Extract, rh EGF, L-glutamine, heparin sulfate, hydrocortisone hemisuccinate, Fetal Bovine Serum, and ascorbic acid
   2. Microvascular Endothelial Cell Growth Kit-VEGF (ATCC PCS-110-041) containing each of the following growth supplements: rh VEGF, rh EGF, rh FGF basic, rh IGF-1, ascorbic acid, L-glutamine, heparin sulfate, hydrocortisone hemisuccinate and Fetal Bovine Serum.

B. Optional media supplements
   1. Gentamicin-Amphotericin B Solution (ATCC PCS-999-025)
   2. Penicillin-Streptomycin-Amphotericin B Solution (ATCC PCS-999-002)
   3. Phenol Red (ATCC PCS-999-001)

C. Reagents for subculture
   1. D-PBS (ATCC 30-2200)
   2. Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) containing 0.05% Trypsin and 0.02% EDTA. **Note: Do not use other trypsin-EDTA concentrations with ATCC PCS-440-0010.**
   3. Trypsin Neutralizing Solution (ATCC PCS-999-004)

Cell Characteristics

Tissue: Neonatal foreskin

Morphology: Cobblestone appearance with large dark nuclei; during proliferation, cells are small and evenly sized, display a high mitotic index and show no presence of smooth muscle cells.

Growth Properties: Adherent

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Preparation of Complete Growth Medium

1. Obtain one growth kit from the freezer; make sure that the caps of all components are tight.
2. Thaw the components of the growth kit just prior to adding them to the basal medium. It is necessary

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to warm the L-glutamine component in a 37°C water bath and shake to dissolve any precipitates prior to adding to the basal medium.

3. Obtain one bottle of Vascular Cell Basal Medium (475 mL) from cold storage.
4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.
5. Using aseptic technique, and working in a laminar flow hood or biosafety cabinet, transfer the volume of each growth kit component, as indicated in either Table 1 or 2, to the bottle of basal medium using a separate sterile pipette for each transfer.

Table 1. If using the Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040), add the indicated volume for each component:

Component	Volume	Final Concentration
Bovine Brain Extract (BBE)	1.0 mL	0.2%
rh EGF	0.5 mL	5 ng/mL
L-glutamine	25.0 mL	10 mM
Heparin sulfate	0.5 mL	0.75 Units/mL
Hydrocortisone hemisuccinate	0.5 mL	1 µg/mL
Fetal Bovine Serum	25.0 mL	5%
Ascorbic acid	0.5 mL	50 µg/mL

Table 2. If using the Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041), add the indicated volume for each component:

Component	Volume	Final Concentration
rh VEGF	0.5 mL	5 ng/mL
rh EGF	0.5 mL	5 ng/mL
rh FGF basic	0.5 mL	5 ng/mL
rh IGF-1	0.5 mL	15 ng/mL
L-glutamine	25.0 mL	10 mM
Heparin sulfate	0.5 mL	0.75 Units/mL
Hydrocortisone hemisuccinate	0.5 mL	1 µg/mL
Fetal Bovine Serum	25.0 mL	5%
Ascorbic acid	0.5 mL	50 µg/mL

Antimicrobials and phenol red are not required for proliferation but may be added if desired. The recommended volume of each ***optional*** component to be added to the complete growth media is summarized in Table 3.

Table 3. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional)

Component	Volume	Final Concentration
Gentamicin-Amphotericin B Solution	0.5 mL	Gentamicin: 10 mg/mL Amphotericin B: 0.25 µg/mL
Penicillin-Streptomycin-Amphotericin B Solution	0.5 mL	Penicillin: 10 Units/mL Streptomycin: 10 µg/mL Amphotericin B: 25 ng/mL
Phenol Red	0.5 mL	33 µM

6. Tightly cap the bottle of complete growth medium and swirl the contents gently to assure a homogeneous solution. Do not shake forcefully to avoid foaming. Label and date the bottle.
7. Complete growth media should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, complete growth media is stable for 30 days.

Handling Procedure for Frozen Cells and Initiation of Culture

1. ***Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC PCS-110-010.***
2. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of 5,000 cells per cm^2.
3. Prepare the desired combination of flasks. Add 5 mL of complete growth media per 25 cm^2 of surface area. Place the flasks in a 37°C, 5% CO_2, humidified incubator and allow the media to pre-equilibrate to

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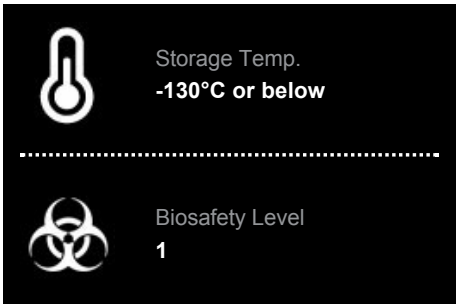

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temperature and pH for 30 minutes prior to adding cells.

4. While the culture flasks equilibrate, remove one vial of ATCC PCS-110-010 from storage and thaw the cells 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 1 to 2 minutes).
5. 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 onward should be carried out under strict aseptic conditions.*
6. Add the appropriate volume of complete growth media [volume = (1 mL x number of flasks to be seeded) - 1 mL] into a sterile conical tube. Using a sterile pipette, transfer the cells from the cryovial to the conical tube. Gently pipette the cells to homogenize the suspension. Do not centrifuge
7. Transfer 1 mL of the cell suspension to each of the pre-equilibrated culture flasks prepared in steps 1 to 3 of Handling Procedure for Frozen Cells and Initiation of Culture. Pipette several times, then cap and gently rock each flask to evenly distribute the cells.
8. Place the seeded culture flasks in the incubator at 37°C with a 5% CO_2 atmosphere. Incubate for at least 24 hours before processing the cells further.

Maintenance

1. Before beginning, pre-warm complete growth media in a 37°C water bath. This will take between 10 and 30 minutes, depending on the volume. If using a small volume of medium (50 mL or less), warm only the volume needed in a sterile conical tube. **Avoid warming complete growth media multiple times.**
2. 24 hours after seeding, remove the cells from the incubator and view each flask under the microscope to determine percent cellular confluence.
3. Carefully remove the spent media without disturbing the monolayer.
4. Add 5 mL of fresh, pre-warmed complete growth media per 25 cm^2 of surface area and return the flasks to the incubator.
5. After 24 to 48 hours, view each flask under the microscope to determine percent cellular confluence. If not ready to passage, repeat steps 3 and 4 as described above. When cultures have reached approximately 80% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture. *Subculture Microvascular Endothelial cells before reaching confluence; post-confluent HDMVECn may exhibit slower proliferation after passaging.* **Note:** Cells are typically ready to passage after 4 to 7 days in culture when inoculated with a seeding density of 5,000 cells/cm^2.

Subculturing

1. Passage normal HDMVECn cells when culture has reached approximately 80% confluence.
2. Warm both the Trypsin-EDTA for Primary Cells (ATCC® PCS-999-003) and the Trypsin Neutralizing Solution (ATCC PCS-999-004) to room temperature prior to dissociation. Warm complete growth medium to 37°C prior to use with the cells.
3. For each flask, carefully aspirate the spent media without disturbing the monolayer.
4. Rinse the cell layer two times with 3 to 5 mL D-PBS (ATCC 30-2200) to remove residual traces of serum.
5. Add pre-warmed trypsin-EDTA solution (1 to 2 mL for every 25 cm^2) to each flask.
6. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer.
7. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 1 to 2 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.
8. When the majority of cells appear to have detached, quickly add an equal volume of Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
9. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the flask.
10. Add 3 to 5 mL D-PBS (ATCC 30-2200) to the flask to collect any additional cells that might have been left behind.
11. Transfer the cell/D-PBS suspension to the centrifuge tube containing the trypsin-EDTA-dissociated cells.
12. Repeat steps 10 and 11 as needed until all cells have been collected from the flask.
13. Centrifuge the cells at 150 x g for 3 to 5 minutes.
14. Aspirate the neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.
15. Count the cells and seed new flasks at a density of 5,000 cells per cm^2.
16. Place newly seeded flasks in a 37°C, 5% CO_2, incubator for at least 24 to 48 hours before processing the cells further. Refer to *Maintenance* for guidelines on feeding.

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Quality Control Specifications

Growth
Each lot of ATCC® PCS-110-010 is tested to ensure the cells will grow for ≥ 15 population doublings after thaw in complete growth media (Vascular Cell Basal Medium plus one Microvascular Endothelial Cell Growth Kit).
Viability: ≥ 50% when thawed from cryopreservation.

Sterility Testing
Bacteria and Yeast: Negative
Mycoplasma: Negative

Viral Testing
Hepatitis B: Negative
Hepatitis C: Negative
HIV-1: Negative
HIV-2: Negative

Specific Staining
Cells are von Willebrand factor positive and smooth muscle alpha-actin negative.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

Human Material Precaution

All tissues used for isolation are obtained under informed consent and conform to HIPAA standards to protect the privacy of the donor's personal health information. It is best to use caution when handling any human cells. We recommend that all human cells be accorded the same level of biosafety consideration as cells known to carry HIV. With infectious virus assays or viral antigen assays, even a negative test result may leave open the possible existence of a latent viral genome.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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