Description

ATCC® Normal Human Primary Epidermal Melanocytes from Neonatal Fore skin, when grown in Dermal Cell Basal Medium supplemented with Melanocyte Growth Kit components, provide an ideal cell system to propagate melanocytes in low serum (less than 1.0% FBS) conditions in the absence of cholera toxin and phorbol 12-myristate 13-acetate (PMA). The cells are cryopreserved in the second passage to ensure the highest viability and plating efficiency. ATCC® Primary Cell Solutions™ cells, media, supplements and reagents are quality tested together to guarantee optimum performance and reliability.

Components:
A. One vial of Primary Epidermal Melanocytes; Normal, Human, Neonatal (ATCC PCS-200-012) containing a minimum of $5 \times 10^5$ viable cells (provided).

Also required:
B. One bottle of Dermal Cell Basal Medium (ATCC PCS-200-030) plus one Melanocyte Growth Kit (ATCC PCS-200-041) that contains the following components: L-glutamine, recombinant human (rh) insulin, ascorbic acid, epinephrine, calcium chloride, and M8 Supplement (a proprietary formulation containing FBS and other factors).

Also Required:
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-201-012.
3. Trypsin Neutralizing Solution (ATCC PCS-999-004)

Cell Characteristics

Morphology: Spindle-shaped; cells are bipolar and refractile.

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 containers are tight.
2. Thaw the components of the growth kit just prior to adding them to the basal medium. It is necessary 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 Fibroblast Basal Medium (480 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 Table 1 or 2, to the bottle of basal medium using a separate sterile pipette for each transfer.
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, media is stable for up to 1 year.
**Dermal Fibroblasts; Normal, Human (ATCC® PCS-201-012™)**

Please read this FIRST

**Storage Temp.**

-130°C or below

**Biosafety Level**

1

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**Intended Use**

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

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**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Dermal Fibroblasts; Normal, Human, Adult (ATCC® PCS-201-012™)

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**Quality Control Specifications**

**Growth**

Each lot of ATCC® PCS-201-012 is tested to ensure the cells will grow for ≥ 10 population doublings after thaw in complete serum-free Fibroblast Media (Fibroblast Basal Medium plus one Fibroblast Growth Kit-Serum-Free).

**Sterility Testing**

Bacteria and Yeasts: Negative.

Mycoplasma: Negative

**Viral Testing**

Hepatitis B: Negative

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**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 to 36 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 medium per 25 cm² 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 80% to 90% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture. Melanocytes are not contact inhibited; however, they will proliferate best if the cells are passaged prior to 100% confluence. Melanocytes from lightly pigmented tissue should reach 80% confluence in 7 to 9 days.

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**Subculturing**

1. Passage normal melanocytes when the culture has reached approximately 80% to 90% 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 the 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 medium.

5. Add pre-warmed trypsin-EDTA solution (1 to 2 mL for every 25 cm²) 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 3 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.

Note: Melanocytes are sensitive to over-trypsinization.

8. When the majority of cells appear to have detached, quickly add an equal volume of the Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipet 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 culture flask.

10. Add 3 to 5 mL D-PBS (ATCC 30-2200) to the tissue culture 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 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 culture flasks at a density of 2,500 to 5,000 cells per cm². Place newly seeded flasks in a 37°C, 5% CO₂ incubator for at least 24 to 48 hours before processing the cells further. Refer to Maintenance for guidelines on feeding.

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**Notes**

- If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Dermal Fibroblasts; Normal, Human, Adult (ATCC® PCS-201-012™).

- Complete growth media is stable for 30 days.

- 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.

- 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 80% to 90% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture. Melanocytes are not contact inhibited; however, they will proliferate best if the cells are passaged prior to 100% confluence. Melanocytes from lightly pigmented tissue should reach 80% confluence in 7 to 9 days.

- When the majority of cells appear to have detached, quickly add an equal volume of the Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipet or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.

- Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.

- Add 3 to 5 mL D-PBS (ATCC 30-2200) to the tissue culture flask to collect any additional cells that might have been left behind.

- Transfer the cell/D-PBS suspension to the centrifuge tube containing the trypsin-EDTA-dissociated cells.

- Repeat steps 10 and 11 as needed until all cells have been collected from the flask.

- Centrifuge the cells at 150 x g for 3 to 5 minutes.

- Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.

- Count the cells and seed new culture flasks at a density of 2,500 to 5,000 cells per cm². Place newly seeded flasks in a 37°C, 5% CO₂ incubator for at least 24 to 48 hours before processing the cells further. Refer to Maintenance for guidelines on feeding.
Hepatitis C: Negative
HIV: Negative

Specific Staining
Positive for L-DOPA Oxidase activity

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 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.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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

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