Primary Epidermal Keratinocytes; Normal, Human, Adult (HEKa)

PCS-200-011™

Description

Primary Epidermal Keratinocytes; Normal, Human, Adult (HEKa) are primary cells that provide a complete solution to propagate keratinocytes isolated from human skin. Use these cells in your research in toxicology, wound repair, skin cancer, response to UV radiation, psoriasis, eczema, viral infection, gene delivery systems, cellular differentiation, and cosmetics research/testing.

Organism: *Homo sapiens*, human
Cell Type: keratinocyte
Tissue: Skin; Epidermis
Age: adult
Gender: Lot-specific
Morphology: Cobblestone appearance; cells are rounded, not flat; cells display a high mitotic index; at near 80% confluence, the cells will be associated with each other in colonies.
Growth properties: Adherent
Disease: Normal
Cells per vial: ≥ 5.0×10^5
Volume: 1.0 mL

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

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

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

All tissues used for isolation are obtained under informed consent and conform to HIPAA regulations to protect the privacy of the donor's Personally Identifiable 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 Human immunodeficiency virus (HIV) and other bloodborne pathogens. With infectious virus assays or viral antigen assays, even a negative test result may not exclude the possibility of the existence of a latent viral genome or infectious viral particles below the lower limit of detection of that assay.

ATCC recommends that appropriate safety procedures be used when handling all primary cells and cell lines, especially those derived from human or other primate material. Handle as a potentially biohazardous material using universal precautions. Cells derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

Unpacking and storage instructions:

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

Complete medium: 1. Obtain one Keratinocyte 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 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 Dermal Cell Basal Medium (485 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 indicated volume of each growth kit component, as indicated in Table 1, 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, complete growth media is stable for 30 days.

Keratinocyte Growth Kit Components:
Bovine Pituitary Extract (BPE), 2.0 mL, 0.4%
rh TGF-a, 0.5 mL, 0.5 ng/mL
L-Glutamine, 15.0 mL, 6 mM
Hydrocortisone Hemisuccinate, 0.5 mL, 100 ng/mL
rh Insulin, 0.5 mL, 5 µg/ml
Epinephrine, 0.5 mL, 1.0 µM
Apo-Transferrin, 0.5 mL, 5 µg/ml

Antimicrobials and phenol red are not required for proliferation but may be added if desired. The recommended volume of either of the optional components (GA solution or PSA solution) to be added to the complete growth media is summarized below.

Table 2. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional):
Gentamicin-Amphotericin B Solution, 0.5 mL, (Gentamicin: 10 µg/mL, Amphotericin B: 0.25 µg/mL) - (Discard, do not use)
Penicillin-Streptomycin-Amphotericin B Solution, 0.5 mL, (Penicillin: 10 Units/mL (Discard, do not use)
Streptomycin: 10 µg/mL, Amphotericin B: 25 ng/mL) (Discard, do not use)
Phenol Red, 0.5 mL, 33 µM

Note: If possible, avoid using antibiotics, as they may inhibit keratinocyte growth.

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-200-011.
3. Trypsin Neutralizing Solution (ATCC PCS-999-004)

Required media: One bottle of Dermal Cell Basal Medium (ATCC PCS-200-030) plus one Keratinocyte Growth Kit (ATCC PCS-200-040) that contains the following growth supplements: Bovine Pituitary Extract (BPE), rh TGF-α, L-glutamine, hydrocortisone hemisuccinate, insulin, epinephrine and apo-transferrin.

Optional media supplements:

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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)

Handling Procedure:

1. ***Refer to the Certificate of Analysis (COA) for the total number of viable cells recovered from this lot of ATCC PCS-200-011.***
2. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density between 5,000 and 8,000 cells per cm^2. ATCC has achieved good results using 6,500 cells/cm^2.
3. Prepare the desired combination of flasks. Add 5 mL of complete growth medium 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 temperature and pH for 30 minutes prior to adding cells.
   Note: The practice of seeding one vial into a single flask often results in overcrowding. At 6,500 cells/cm^2, one vial (assuming ≥ 500,000 cells) can be distributed into three 25 cm^2 flasks or one 75 cm^2 flask.
   Note: Thicker plastic (e.g., Corning flasks) is preferable. Thinner plastics (e.g., Falcon) may cause static issues
4. While the culture flasks equilibrate, remove one vial of ATCC PCS-200-011 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 medium [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.
   Note: Contrary to previous guidance, gentle centrifugation (e.g., 150xg as per protocol) does not negatively impact cell quality. ATCC have done that on regular basis.
7. Transfer 1.0 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 gently several times, then cap and gently rock each flask to evenly distribute the cells.
   Note: Relying solely on the Certificate of Analysis (COA) for cell number

without actual counting is not recommended.

8. Place the seeded culture flasks in the incubator at 37°C, 5% CO_2 atmosphere. Incubate for at least 24 hours before processing the cells further.

Subculturing procedure:

1. Passage normal keratinocytes when the culture has reached approximately 70% to 80% confluence.
   Note: Do not exceed 80% confluence. High confluence can lead to differentiation and make trypsinization difficult due to strong adherence.
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 one time 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 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 3 to 6 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.
   If cells are difficult to detach, incubate each flask containing cells and the trypsin-EDTA solution at 37°C to facilitate dispersal.
   Note: Avoid over-trypsinizing. A maximum of 10 minutes is advised; prolonged exposure may indicate differentiation. If needed, re-trypsinization of the adhered cells is an option, though cells may already be suboptimal at that point.
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 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 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 cm^2.
    Note: To support expansion, avoid overseeding.
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.

Culture 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 medium 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. *Keratinocytes will begin to terminally differentiate once they become 100% confluent.*

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Primary Epidermal Keratinocytes; Normal, Human, Adult (HEKa) (ATCC PCS-200-011)

References

References and other information relating to this material are available at www.atcc.org.

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