

Human Epidermal Keratinocyte-adult (HEK-a)

Catalog Number	10HU-225	Cell Number	0.5 million cells/vial
Species	<i>Homo sapiens</i>	Storage Temperature	Liquid nitrogen

Description

Epidermal keratinocyte is the predominant cell type in the outermost layer of the skin - the epidermis, which serves as a critical barrier to separate and protect the inside of human body from outside environment and damage from pathogens, heat, UV radiation and water loss. Epidermal keratinocytes originate in the stratum basal, and they undergo gradual differentiation and migrate towards the surface of the epidermis until they reach the stratum corneum, where they form a tight layer of nucleus-free and highly keratinized squamous cells. This layer forms an effective barrier to prevent water loss and the entry of infectious agents [1,2]. Keratinocytes are also known to produce various growth factors, cytokines, antimicrobial peptides, and complement factors. Therefore, keratinocytes are important for wound healing, inflammation, infection, skin microbiome and immune response [3].

iXCells Biotechnologies provides high quality primary Human Epidermal Keratinocytes-adult (HEK-a), which are isolated from adult human skin and cryopreserved at P1, with ≥ 0.5 million cells in each vial. HEK-a are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. They can further expand no more than 3 passages in Keratinocyte Growth Medium (Cat# MD-0047) under the condition suggested by iXCells Biotechnologies.

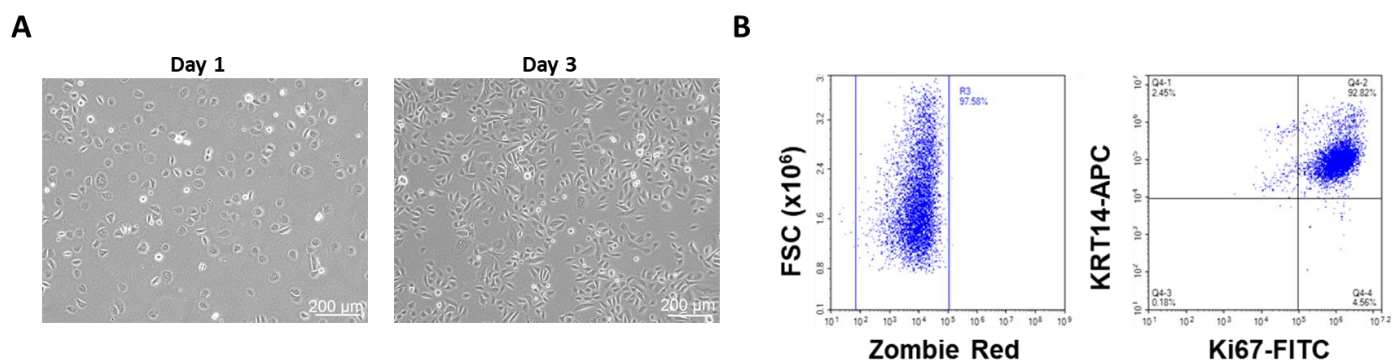


Figure 1. (A). Phase contrast image of primary Human Epidermal Keratinocytes - Adult (HEK-a). (B). HEK-a cells were collected 3 days post recovery and incubated with Zombie Red to distinguish live and dead cells followed by fixation using 70% ethanol overnight. Keratinocytes were then stained with Ki67 and cytokeratin 14. Live cells were gated using Zombie Red first, and proliferating Keratinocytes were shown as Ki67+KRT14+.

Product Details

Tissue	Epidermis from adult human skin
Package Size	0.5 million cells/vial
Passage Number	P1
Shipped	Frozen
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Keratinocyte Growth Medium (Cat# MD-0047)

Protocols

Coating Protocol using Collagen I

1. Dilute Collagen I (Corning, Cat#354236) with distilled water to 100 µg/mL.
2. Add sufficient diluted collagen I to the desired culture vessel. We recommend 1 mL/well in 6-well culture plate.
3. Incubate the coating for 1 hour at 37°C.
4. Remove remaining solution and rinse wells with PBS 3 times to remove acid.
5. Aspirate the PBS and use the plate immediately, or air dry the plates and store the coated plate at 2-8 °C

Note: 1) Coating the culture vessels is required.

2) The Collagen I from other vendors should also work well. Please perform the test before the large scale of experiment.

Thawing of Frozen cells

1. Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately to retain the highest cell viability.
2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for 1-2 minutes. Keep the cap out of water to minimize the risk of contamination.
3. Pipette the cells into a 15 mL conical tube with 5 mL fresh **Keratinocyte Growth Medium** (Cat# MD-0047).
4. Centrifuge at 600g for 5 minutes under room temperature.
5. Remove the supernatant and resuspend the cells in fresh culture medium.
6. Culture the cell at 10,000 cells-20,000/cm² using the collagen I-precoated culture vessels in a 37°C, 5% CO₂ incubator. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.
7. Change the culture medium every other day until the culture is approximately 50% confluent. Then change the medium daily until the cells reach the desired confluency prior to the experiment.

Note: Upon thawing of the cells, please plate cells as soon as possible because prolonged incubation in suspension will lead to terminal differentiation of keratinocytes.

Standard culture procedure

1. HEK-a can be cultured in **Keratinocytes Growth Medium** (Cat# MD-0047).
2. When cells reach ~80-90% confluence, remove the medium, and wash once with sterile PBS (1 mL/well in 6-well plate or 5 mL/T75 flask).
3. Add 1 mL/well of TrypLE™ Select Enzyme (Thermo Fisher, Cat# 12563029) in the 6-well plate and incubate for 3-5 minutes at 37°C.
4. Collect detached the cells by thoroughly rinsing the culture vessels using 2-3 volumes of cell culture medium.
5. Centrifuge 600 g for 5 minutes and resuspend the cells in desired volume of medium.
6. Seed the cells at 10,000 cells/cm² onto the new culture vessels precoated with Collagen I. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.
7. Change the culture medium every other day until the culture is approximately 50% confluent. Then change the medium daily until the cells reach the desired confluency prior to the experiment.

Note: *The unattached cells may be noted after overnight seeding. Please refer the CoA of each product Lot for the Lot specific growth rate due to the donor variations.*

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Reference

- [1] Raja, Sivamani K, Garcia MS, Isseroff RR. Wound re-epithelialization: modulating keratinocyte migration in wound healing. Front Biosci. 2007 May 1;12:2849-68.
- [2] Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol. 2008 Dec;17(12):1063-72.
- [3] Pasparakis M, Haase I, Nestle FO. Mechanisms regulating skin immunity and inflammation. Nat Rev Immunol. 2014 May;14(5):289-301.

Disclaimers

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