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Product Information

Human Epidermal Keratinocyte-adult (HEK-a)

Catalog Number	10HU-225	Cell Number	0.5 million cells/vial
Species	*Homo sapiens*	Storage Temperature	Liquid Nitrogen

Description

A keratinocyte is the predominant cell type in the epidermis, constituting 90% of the cells found there [1]. The primary function of keratinocytes is the formation of a barrier against environmental damage by pathogenic bacteria, fungi, parasites, viruses, heat, UV radiation and water loss. Keratinocytes are also able to produce a variety of cytokines, growth factors, interleukins and complement factors. Therefore keratinocytes are important for wound healing, inflammation, and immune response.

iXCells Biotechnologies provides high quality Human Epidermal Keratinocyte-adult (HEK-a), which are isolated from adult skin samples 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.

Product Details

Tissue	Adult skin
Package Size	0.5 million cells/vial
Passage Number	P1
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Keratinocyte Growth Medium (MD-0047)

Protocols

Coating protocol

1. Prepare the Coating Matrix Kit (Thermo Fisher, Cat#R-011-K) according to the manual's instruction;
2. Dilute 100 µl Coating Matrix (Thermo Fisher, Cat#50-9700) in 10 mL Dilution Medium (Thermo Fisher, Cat#50-9701) at 1:100, and mix well;
3. Add 5ml mixed coating matrix solution per each T75 flask;
4. Rock back and forth to ensure uniform distribution of the coating matrix over the surface of the flask;
5. Cap the flasks and incubate for 30 minutes at room temperature;
6. Remove excess Coating Matrix/Dilution Medium from each flask. The flasks may be used immediately, or may be stored at 2° to 8° C for short periods.

Thawing of Frozen Cells

1. Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately in order 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 minute. 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** (MD-0047).
4. Centrifuge at 1,000 rpm (~220 g) for 5 minutes under room temperature.
5. Remove the supernatant and resuspend the cells in fresh culture medium.
6. Culture the cell in T75 flask precoated with Coating Matrix (Thermo Fisher Scientific, Cat# R011K) in a 37°C CO_2 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 every day until the culture is approximately 80% confluent.

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

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

Standard Culture Procedure

1. Keratinocytes can be cultured in **Keratinocyte Growth Medium** (MD-0047).
2. When cells reach ~80-90% confluence, remove the medium, and wash once with sterile PBS (5ml/T75 flask).
3. Add ~2.5 mL of 0.25% TrypLE Selection (Thermo Fisher, Cat#12563029) to the flask and incubate for ~3 minutes at 37°C. Neutralize by adding 2-3 volumes of Keratinocyte Growth Medium.
4. Centrifuge 1,000 rpm (~220 g) for 5 minutes and resuspend the cells in desired volume of Keratinocyte Growth Medium.
5. Seed the cells onto new culture vessels at 3×10^3 cells/cm^2.
6. Change the culture medium every other day until the culture is approximately 50% confluent. Then change the medium every day until the culture is approximately 80% confluent.

Reference

[1] McGrath JA, Eady RAJ, Pope FM. (2004). "Anatomy and Organization of Human Skin". In Burns T, Breathnach S, Cox N, Griffiths C. Rook's Textbook of Dermatology (7th ed.). Blackwell Publishing. p. 4190.

Disclaimers