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Mouse Epidermal Keratinocytes – Adult (MEK-a)

Catalog Number	10MU-035	Cell Number	0.5 million cells/vial
Species	*Mus Musculus*	Storage Temperature	Liquid nitrogen

Product 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 basale, 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 Mouse Epidermal Keratinocytes-adult (MEK-a), which are isolated from adult mouse tail and cryopreserved at P0, with ≥0.5 million cells in each vial. MEK-a are characterized by phalloidin staining and are negative for mycoplasma, bacteria, yeast and fungi. ***MEK-a are not recommended for expanding or long-term cultures.***

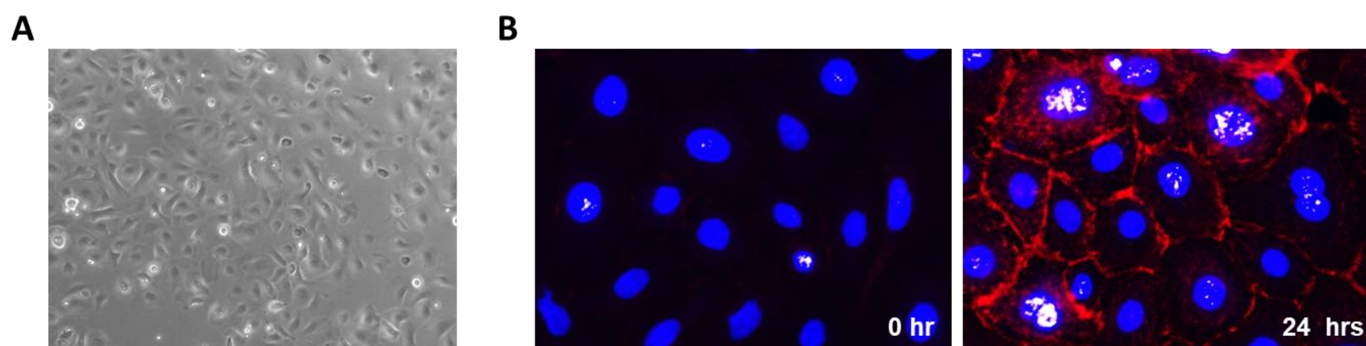

Figure 1. (**A**). Phase contrast image of primary Mouse Epidermal Keratinocytes - Adult (MEK-a). (**B**). MEK-a were differentiated in the presence of 0.2mM $CaCl_2$, and cells were stained with phalloidin (Red) to visualize the formation of actin fiber-rich filopodial projections between adjacent cells during differentiation. Nuclei were counterstained with DAPI.

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Product Details

Tissue	Epidermis from adult mouse tail
Package Size	0.5 million cells/vial
Passage Number	P0
Shipped	Frozen
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Keratinocyte Growth Medium (Cat# MD-0047)

Protocols

Coating Protocol using Collage I

1. Dilute Collage I (Corning, Cat#354236) with 0.01M HCl to 50 µg/mL;
2. Add sufficient diluted collagen to the desired culture vessel. We recommend adding 1 mL/well in 6-well culture plate;
3. Incubate the coating for 1 hour at room temperature;
4. Remove remaining solution and rinse wells with PBS or culture medium to remove acid;
5. Use the plate immediately, or air dry the plates and store the coated plate at 2-8 °C

Coating Protocol with Coating Matrix Kit (Invitrogen, Catalog# R011K)

1. Dilute 100µl Coating Matrix (50-9700) in 10ml Dilution Medium (50-9701) at 1:100, and mix well;
2. Add 5ml mixed coating matrix solution per each T75 flask;
3. Rock back and forth to ensure uniform distribution of the coating matrix over the surface of the flask;
4. Cap the flasks and incubate for 30 minutes at room temperature;
5. 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.

   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 and standard culture procedure

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 minutes. Keep the cap out of water

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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 350 *g* 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/cm^2 in 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 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.*

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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