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Product Information

Mouse Epidermal Keratinocytes – neonatal (MEK-n)

Catalog Number	10MU-020	Cell Number	0.5 million cells/vial; 1.0 million cells/vial
Species	*Mus Musculus*	Storage Temperature	Liquid nitrogen; or put into incubator immediately upon delivery

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. 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.

iXCells Biotechnologies provides high quality primary Mouse Epidermal Keratinocytes-neonatal (MEK-n), which are isolated from neonatal mouse skin and cryopreserved at P0, with >0.5 million or >1 million cells in each vial. MEK-n are characterized by phalloidin staining and are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. ***MEK-n are not recommended for expanding or long term cultures since the cells do not proliferate in culture.***

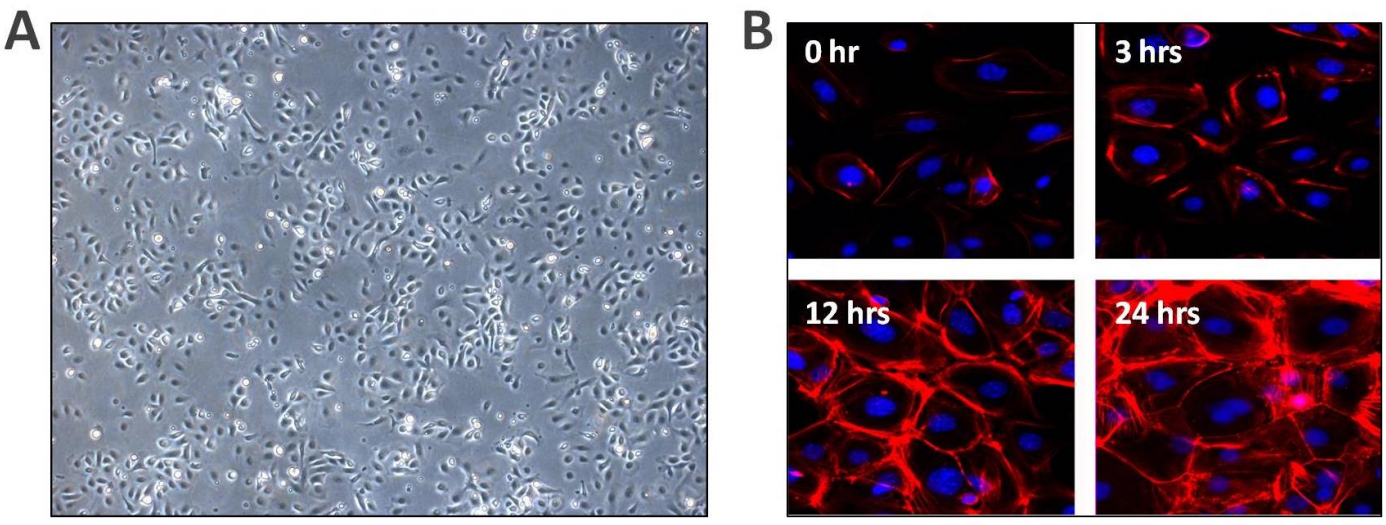

Figure 1. (**A**). Phase contrast image of primary neonatal mouse epidermal keratinocytes (MEK-n). (**B**). MEK-n were differentiated in the presence of 0.2mM $CaCl_2$, and cells were stained with phalloidin (red) to visualize the formation of actin fiber-rich filopodial projections between adjacent cells during differentiation. Nuclei were counterstained with DAPI.

Product Details

Tissue	Epidermis from mouse neonatal skin
Package Size	1 x10^6 cells/vial (Enough to seed on four wells of 6-well culture plate or eight wells of 12-well culture plate, and cells will reach 80% confluency in 5 days for assay). 0.5 x10^6 cells/vial
Passage Number	P0
Shipped	Frozen
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Keratinocyte Growth Medium (Cat# MD-0047)

Protocols

Optional: Prepare culture vessels with Coating Matrix Kit (Invitrogen, Catalo# R011K).

1. Using sterile technique in a laminar flow culture hood, add Dilution Medium to each well (1 mL per 6-well or 0.5 mL per well of 12-well culture plate).
2. Add Coating Matrix (100X) directly to the Dilution Medium in each culture vessels (10 µL per well of the 6-well culture plate; or 5 µL per well of the 12-well culture plate). Rock back and forth vigorously to ensure uniform distribution of the coating matrix over the surface of the culture vessels.
3. Incubate for 30 minutes at room temperature.
4. Remove excess Coating Matrix/Dilution Medium from each well. The flasks may be used immediately, or may be stored at 2° to 8° C for about a week.

Note: *Coating the culture vessels is optional, but highly recommended.*

Instruction for Mouse Epidermal Keratinocytes Culture

1. Prepare the complete **Keratinocyte Growth Medium (Cat# MD-0047)** by adding 5mL 100X supplement into 500mL basal medium. The complete medium can be stored at 4°C for 1 month after the addition of supplement. The complete medium should **NOT** be **frozen**.
2. Warm up 9 mL of complete medium in a 15 mL falcon tube.
3. Remove one vial of frozen cells from liquid nitrogen.
4. Thaw the vial in a 37°C water bath with gentle swirling for 1-2 minutes.
5. Wipe down the vial with ethanol and tap gently on a surface so that all the medium collects at the bottom of the tube. Open the vial in a laminar flow hood.
6. Transfer 1mL pre-warmed complete medium from Step1 to the vial, and gently pipet up and down the cells. Avoid creating any air bubbles.
7. Transfer all the vial content (total 2 mL) to the 6 mL pre-warmed culture medium in a 15 mL falcon tube.
8. Mix gently by inverting a few times.
9. Plate 2mL (~5x10^5) cells per well of the pre-coated 6-well culture plate or 1 mL to each well of 12-well culture plate.
10. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
11. Feed the cells every other day with fresh complete medium. Cells should reach 80% confluency within 5 days before assay.

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.*

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References

[1] Raja, Sivamani K, Garcia MS, Isseroff RR. *Front Biosci.* 2007;12:2849-68. Wound re-epithelialization: modulating keratinocyte migration in wound healing.

[2] Proksch E1, Brandner JM, Jensen JM. *Exp Dermatol.* 2008 Dec;17(12):1063-72. The skin: an indispensable barrier.

[3] Pasparakis M, Haase I, Nestle FO. *Nat Rev Immunol.* 2014 May;14(5):289-301. Mechanisms regulating skin immunity and inflammation.

Disclaimers