Rev. 1

Rat Epidermal Keratinocytes (REK)

Catalog #R2100

Cell Specification

The epidermal layer of the skin provides an essential function as a protective barrier against insults from the external environment. The predominant cell type in the epidermis is keratinocytes which account for around 85% of living epidermal cells. They are so named because the most abundant protein in this cell type is keratin. Keratinocytes belong to stratified squamous epithelia. Progenitors of keratinocytes reside and divide in the basal layer of the epidermis. They then differentiate, migrate towards the surface of epidermis, and eventually withdraw from the cell cycle permanently. Keratinocyte proliferation, differentiation, and programmed cell death are complex and carefully choreographed processes [1]. Apart from their protective functions, keratinocytes express adhesion molecules and cytokines, further suggesting an implication in skin innate immunity, tissue homeostasis, wound healing, cancer development, and skin-based gene-therapy [2, 3].

REK from ScienCell Research Laboratories are isolated from neonatal rat skin. REK are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. REK are characterized by immunofluorescence with antibodies specific to cytokeratin-18. REK are negative for mycoplasma, bacteria, yeast, and fungi. REK are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; *however, REK are not recommended for long-term cultures due to limited expansion capacity and senescence after subculturing.*

Recommended Medium

It is recommended to use Keratinocyte Medium (KM, Cat. #2101) for culturing REK *in vitro*.

Product Use

REK are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Eckert RL, Efimova T, Dashti SR, Balasubramanian S, Deucher A, Crish JF, Sturniolo M, Bone F. (2002) "Keratinocyte survival, differentiation, and death: many roads lead to mitogen-activated protein kinase." *J Investig Dermatol Symp Proc*. 7: 36-40.

[2] Song PI, Park YM, Abraham T, Harten B, Zivony A, Neparidze N, Armstrong CA, Ansel JC. (2002) "Human keratinocytes express functional CD14 and toll-like receptor 4." *J Invest Dermatol*. 119: 424-32.

[3] de Panfilis G, Semenza D, Lavazza A, Mulder AA, Mommaas AM, Pasolini G. (2002) "Keratinocytes constitutively express the CD95 ligand molecule on the plasma membrane: an in situ immunoelectron microscopy study on ultracryosections of normal human skin." *Br J Dermatol*. 147: 7-12.

Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-$_L$-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-$_L$-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add 20 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-$_L$-lysine-coated culture vessel. A seeding density of 7,000-9,000 cells/cm^2 is recommended.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-$_L$-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every 2-3 days thereafter.

Note: Rat Epidermal Keratinocytes are not recommended for long-term culture due to limited expansion capacity and senescence after subculturing.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

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Caution: Handling animal-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.