ScienCell™
Research Laboratories

Human Epidermal Keratinocytes (HEK)

Catalog Number: 2100, 2110, 2120

Cell Specification

The epithelial layer of the skin provides an essential function as a protective barrier against insult from the outside environment. The major cell type in this layer is keratinocytes which consists around 85% of living epidermal cells. Kertinocytes are the cells of stratified squamous epithelia. It is so named because its most abundant protein is keratin. Keratinocyte divide and are thus generated in the basal layer of the epidermis; this division is followed by a programmed death as they produce keratin and move up to the surface of the epidermis. Keratinocyte proliferation, differentiation, and apoptosis are a complex and carefully choreographed process [1]. Besides the protective function, keratinocyte also plays a central role in tissue homeostasis, wound healing, cancers, and skin-based gene-therapy. Human keratinocytes express adhesion molecules and cytokines indicating their participation in skin innate immunity and homeostasis *in vivo* [2, 3].

HEK from ScienCell Research Laboratories are isolated from human epidermal tissue. HEK are cryopreserved on primary culture and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HEK are characterized by immunofluorescent method with antibodies to cytokeratine-8, -18 and -19. HEK are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HEK are guaranteed to further expand for 15 population doublings in the condition provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use keratinocyte medium (KM, Cat. No. 2101) for the culturing of HEK *in vitro*.

Product Use

HEK are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments.

Shipping

Dry ice.

Reference

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

[2] Song, P. I., Park, Y. M., Abraham, T., Harten, B., Zivony, A., Neparidze, N., Armstrong, C. A. and Ansel, J. C. (2002) Human keratinocytes express functional CD14 and toll-like receptor 4. *J Invest Dermatol* 119(2):424-32.

[3] de Panfilis, G., Semenza, D., Lavazza, A., Mulder, A. A., Mommaas, A. M. and 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(1):7-12.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37ºC waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (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, ScienCell cat. no. 0413). Leave flask in incubator overnight (minimum one hour at 37ºC incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 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 DMSO residue in the culture. It is also important that keratinocytes are plated in poly-L-lysine coated culture vessels that promote cell attachment.
6. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display cobblestone morphology, nongranular cytoplasm, and the cell number will be double after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks (2 μg/cm^2).
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) for 5 min at 37°C or until cells are completely rounded up (monitored with microscope). Remove trypsin/EDTA solution and further incubate the cells at 37°C for 1 min. With one hand hold the flask, gently tap the edge of flask with the other hand to release cells from the culture surface. Add 10 ml of trypsin neutralization solution to flask.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Transfer the released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working 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, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).