Rev.0

Bovine Lens Epithelial Cells (BLEpiC)

Catalog #B6550

Cell Specification

The mammalian lens consists of two cell types, lens fiber cells which form the bulk of the lens, and a monolayer of epithelial cells that cover the anterior surface of the fibers. Lens epithelial cells (LEpiC) are responsible for homeostasis regulation of the lens, including electrolyte and fluid transport [1]. Under normal development, LEpiC progressively differentiate and mature. LEpiC then migrate from the equatorial region into the interior of the lens to produce transparent crystallins, elongate to form lens fiber cells, and eventually lose their nuclei and other organelles [2]. Studies have shown that LEpiC differentiation and lens polarization are regulated by growth factors present in the ocular fluids [3], such as epidermal growth factor, basic fibroblast growth factor, insulin growth factor, and insulin [4]. Misregulation of LEpiC leads to the development of ocular diseases such as cataracts. Bovine LEpiC (BLEpiC) can be used to eludicate the mechanisms involved in the opacification of the lens.

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

Recommended Medium

It is recommended to use Epithelial Cell Medium-animal (EpiCM-a, Cat. #4131) for culturing BLEpiC *in vitro*.

Product Use

BLEpiC 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] Candia OA. (2004) "Electrolyte and fluid transport across corneal, conjunctival and lens epithelia." *Exp Eye Res*. 78: 527-35.

[2] Wagner LM, Takemoto DJ. (2001) "PKCα and PKCγ overexpression causes lentoid body formation in the N/N 1003A rabbit lens epithelial cell line." *Molecular Vision*. 7: 138-44.

[3] Lang RA. (1999) "Which factors stimulate lens fiber cell differentiation in vivo?" *Invest Opthalmol Vis Sci*. 40: 3075-8.

[4] Leenders WP, van Genesen ST, Schoenmakers JG, van Zoelen EJ, Lubsen NH. (1997) "Synergism between temporally distinct growth factors: bFGF, insulin and lens cell differentiation." *Mech Dev*. 67: 193-201.

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Instructions for culturing primary 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.

Note: *Experiments should be well organized before thawing BLEpiC. It is recommended that BLEpiC are used for experiments as quickly as possible after thawing the cells.* ***BLEpiC should not be subcultured or passaged, as the cells have a limited expansion capacity.***

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 25 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 10,000-15,000 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 48 hours after the culture has been initiated in order to allow cells to attach. Carefully remove the culture medium after 48 hours to remove residual DMSO, then every 2 to 3 days thereafter.
9. Use cells as soon as possible for experiments once the cells become confluent.

Note: Bovine Lens Epithelial Cells 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.

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.