Rev. 0

Rabbit Iris Pigment Epithelial Cells (RabIPEpiC)

Catalog #Rab6560

Cell Specification

The iris is a pigmented disk with a variable aperture which controls the size of the pupil and the amount of light reaching the retina. It consists of the anterior limiting layer, the stroma, the dilator muscle layer, and the posterior pigmented epithelium. Iris pigment epithelial cells (IPEpiC) share the same embryonic origin as retinal pigment epithelial cells (RPE) and thus exhibit similar functional properties as RPE, including turnover of photoreceptors and synthesis of trophic factors [1]. Studies show that IPEpiC transplanted into the subretinal space inhibited abnormal neovascularization and photoreceptor degeneration, which suggests that IPEpiC transplantation may be used in the future to treat retinal disorders [2]. IPEpiC may also have potential to be used as a cell source for the treatment of neurodegenerative diseases.

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

Recommended Medium

It is recommended to use Epithelial Cell Medium (EpiCM, Cat. #4101) for culturing RabIPEpiC *in vitro*.

Product Use

RabIPEpiC 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] Schraermeyer U, Enzmann V, Kohen L, Addicks K, Wiedemann P, Heimann K. (1997) "Porcine iris pigment epithelial cells can take up retinal outer segments." *Exp Eye Res*. 65: 277-87.

[2] Semkova I, Kreppel F, Welsandt G, Luther T, Kozlowski J, Janicki H, Kochanek S, Schraermeyer U. (2004) "Autologous transplantation of genetically modified iris pigment epithelial cells: a promising concept for the treatment of age-related macular degeneration and other disorders of the eye." *PNAS*. 99: 13090-5.

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.**

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). To obtain a 2 µg/cm^2 poly-$_L$-lysine-coated culture vessel, 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 5,000-7,000 cells 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. Do not disturb the culture for at least 16 hours after initiation. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.
4. Use cells promptly for experiments.

   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.