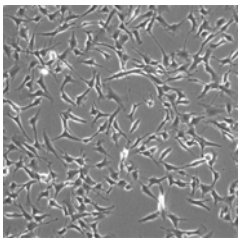
Innoprot
Speeding up drug discovery

REF: P10860

OCULAR CELL SYSTEM INNOPROFILE™
HUMAN IRIS FIBROBLASTS



Product Type:	Cryo-preserved Iris Fibroblasts
Catalog Number:	P10860
Source:	Human Iris
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Iris Fibroblast (HIrF) provided by Innoprot are isolated from healthy iris. HIrF are cryopreserved at primary culture and delivered frozen. HIrF are guaranteed to further expand for 15 population doublings at the condition provided in the technical sheet.

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 fibroblasts (IrF), which are located in the iris, are mesenchymal cells derived from the embryonic mesoderm. The main functions of IrF are to maintain the structural integrity of the connective tissue and to aid in tissue repair and remodeling. Under disease conditions, such as with rubeosis iridis, neovascularization can occur on the anterior surface of the iris and result in fibrosis. If the rubeosis iridis condition is left untreated, a neovascular glaucoma can develop. Patients with diabetes and diabetic retinopathy are at a higher risk of developing rubeosis iridis [4]. Human IrF (HIrF) cultures can be used as an in vitro model for studying fibrosis and associated disorders.

Recommended Medium

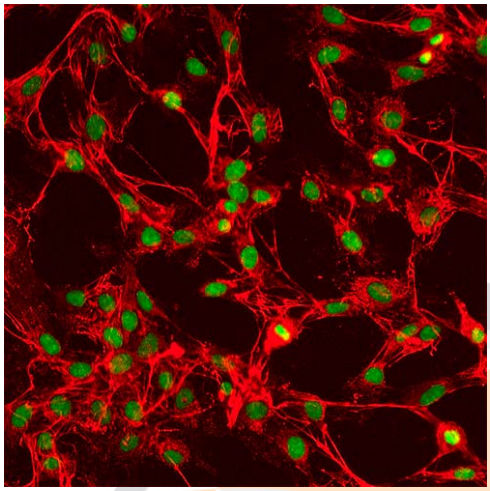
- Fibroblast Medium
  (Reference: P60108)

Product Characterization

Immunofluorescent method

- Fibronectin

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi



Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC 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/cm², T-75 flask is recommended) and leave the 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 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/cm² 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 cells are plated in poly-L-lysine coated culture vessels that promote fibroblast attachment.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps 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 stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be doubled 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

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Subculture:

1. Subculture the cells when they are over 90% confluent.

2. Prepare poly-L-lysine coated cell culture flasks.

3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37ºC 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) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

6. Harvest and transfer 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).

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