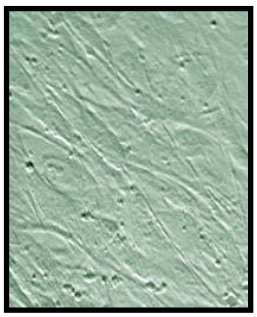
REF: P10883

HAIR CELL SYSTEM INNOPROFILE™

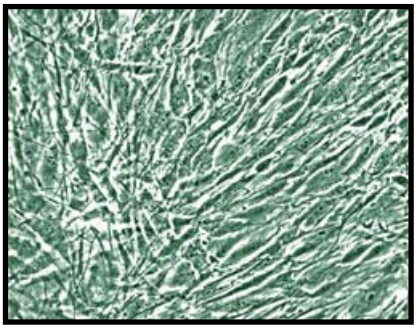
HUMAN HAIR FOLLICLE OUTER ROOT SHEATH CELLS



Product Type:	Cryo-preserved Outer Root Sheath Cells
Catalog Number:	P10883
Source:	Hair Follicle Ourter Root Sheath
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Hair Follicle Outer Root Sheath Cells (HHFORSC) provided by Innoprot are isolated from human hair follicle ourter root sheath. HHFORSC are cryopreserved at primary culture and delivered frozen. HHFORSC are guaranteed to further expand for 10 population doublings in the conditions described in the technical sheet.

The outer root sheath (ORS) of the hair follicle surrounds the hair fiber and inner root sheath. It is distinct from other epidermal components being continuous with the surface epidermis. The ORS consists of several layers of cells that can be identified with unique ultrastructural properties; plays a part in certain function of the hair follicle which including acts as the sensory organ and immunologic sentinel of the skin. Immunohistochemical staining indicates that the ORS contains nestin-expressing progenitor or stem cells. Human outer root sheath cells represent a repeatedly available source of keratinocytes which can be easily and extensively expanded in culture.



Recommended Medium

- Mesenchymal Stem Cell Medium (Reference: P60115)

Product Characterization

Immunofluorescent method

- CD105
- 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 °C 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). Leave the flask in incubator overnight (minimum one hour at 37°C 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 37°C 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 re-suspend 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 HHFORSC are plated in poly-L-lysine coated flask that promotes cell attachment and growth.

6. Replace the cap or cover, 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.

Maintenance of Culture:

1. Change the medium to fresh supplemented 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% confluence, change medium every other day until the culture is approximately 90% 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 (2 µg/cm^2).

3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS (Ca++ and Mg++ free) 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. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum; transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37°C for 1 or 2 minutes more (no solution in the flask at this moment); at the end of trypsinisation, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (Beckman Coulter Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.

7. Count cells and plate cells 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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