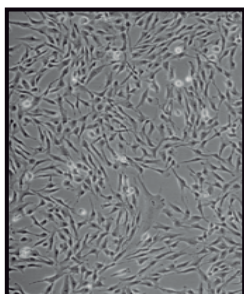


TONSIL CELL SYSTEM INNOPROFILE™ HUMAN TONSIL FIBROBLASTS



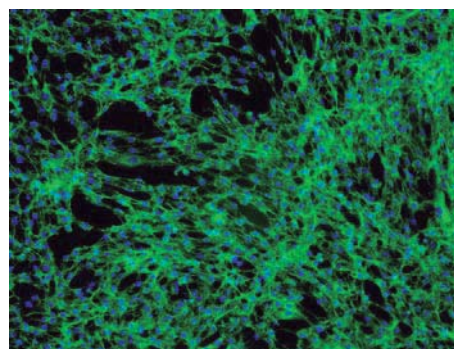
Product Type:	Cryo-preserved Fibroblasts
Catalog Number:	P10888
Source:	Human Tonsil
Number of Cells:	5 x 10 ⁵ Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Tonsil Fibroblasts (HTF) provided by Innoprot are isolated from human tonsil. HTF are cryopreserved at passage one and delivered frozen. HTF are guaranteed to further expand for 15 population doublings in the conditions provided in the technical sheet.

Fibroblasts are mesenchymal cells derived from the embryonic mesoderm. They have been extensively used for a wide range of cellular and molecular studies. This is mainly because they are one of easiest types of cells to grow in culture, and their durability makes them amenable to a wide variety of manipulations ranging from studies employing gene transfection to microinjection. Fibroblasts secrete a nonrigid extracellular matrix that is rich in type I and/or type III collagen. They are responsible for much of the synthesis of extracellular matrix in connective tissues and play major roles in wound healing. Many diseases are associated with fibroblasts, either because fibroblasts are implicated in their etiology or because of the fibrosis that accompanies damage to other cell types in tissues.

Recommended Medium

- Fibroblasts 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

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 $\mu\text{g}/\text{cm}^2$, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 150 μl of poly-L-lysine stock solution (1 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 bottle and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated vessel with sterile water twice and then add 15 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 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to a sterile field.
5. Remove the cap carefully 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 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 HTF 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 the best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture 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

Subculture:

1. Subculture when the culture reaches 90% confluency or above.
2. Prepare poly-L-lysine-coated culture vessels ($2 \mu\text{g}/\text{cm}^2$) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, Trypsin Neutralization Solution, and DPBS (Ref: P60305 - Primary Cells Subculturing Kit) to room temperature. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another 1 to 2 minutes (no solution in the flask at this moment).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must 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).