Human Dermal Fibroblasts-Fetal-Mitomycin C treated (HDF-f-mt)

Catalog #2350

Cell Specification

Fibroblasts are mesenchymal cells derived from the embryonic mesoderm. They have been extensively used for a wide range of cellular and molecular studies as they are one of easiest types of cells to grow in culture. Their durability also makes them amenable to a variety of manipulations ranging from studies employing gene transfection to microinjection. In general, fibroblasts secrete a non-rigid extracellular matrix which is rich in type I and/or type III collagen [1]. There is evidence showing that fibroblasts in various organs are intrinsically different [2]. Dermal fibroblasts switch from a proliferative, migratory phase to a contractile, matrix-remodeling phase during wound healing. In addition, they secrete large quantities of hyaluronan in response to inflammatory stimuli [3].

HDF-f-mt from ScienCell Research Laboratories are isolated from fetal human skin. HDF-f-mt are cryopreserved at P0 and delivered frozen. Each vial contains > 5 x 10^5 cells in 1 ml volume. HDF-f-mt are characterized by their spindle morphology and immunofluorescence with antibody specific to fibronectin. HDF-f-mt are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HDF-f-mt are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; *however, HDF-f-mt are not recommended for expanding as mitomycin C treatment prevents further cell proliferation*.

Recommended Medium

It is recommended to use Fibroblast Medium (FM, Cat. #2301) for culturing HDF-f-mt *in vitro*.

Product Use

HDF-f-mt 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] Gabbiani G, Rungger-Brandle E. (1981) "The fibroblast." In Glynn LE, *Handbook of Inflammation, Vol. 3: Tissue Repair and Regeneration* (pp 1-50). Amsterdam: Elsevier.

[2] Conrad GW, Hart GW, Chen Y. (1977) "Differences in vitro between fibroblast-like cells from cornea, heart, and skin of embryonic chicks." *J Cell Sci*. 26: 119-37.

[3] Stair S, Carlson KW, Shuster S, Wei ET, Stern R. (2002) "Mystixin peptides reduce hyaluronan deposition and edema formation." *Eur J Pharmacol*. 450: 291-6.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling!

Initiating the culture:

1. Prepare 0.1% gelatin (Cat. #0423) coated culture vessel. Use enough volume of gelatin to cover the entire culture surface. Leave the vessel in a 37°C incubator for 1 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 tube with medium to recover the entire volume.
3. Completely aspirate gelatin from the coated vessel. It is not necessary to rinse the vessel. Add complete medium to cover the culture surface. 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. 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 a 15 ml conical centrifuge tube. Add appropriate volume of complete medium to the tube, gently mix well and plate cells into the equilibrated, gelatin-coated culture vessel at required plating density.

   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 gelatin-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 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells. Cells are then ready for experiments as feeder layer.

HDF-f-mt are not recommended to be subcultured as mitomycin C treatment prevents further cell proliferation.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests 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 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.