NEUROMICS

Human Dermal Fibroblasts – Type 1 Diabetes Donor

Catalog #: HDF002

Cell #: >0.5x10^5 cells

Storage: Liquid Nitrogen until ready for culture.
While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen Vial

GENERAL INFORMATION

Human Dermal Fibroblasts from Type 1 Diabetes donors are isolated from the dermal layer of skin tissue obtained from donors with a clinically confirmed diagnosis of Type 1 Diabetes Mellitus. These cells are cryopreserved at the earliest possible passage to preserve their in vivo-like characteristics and disease-specific phenotype.

HDFs are the principal architects of the extracellular matrix (ECM) and play a critical role in wound healing, inflammation, and tissue integrity. HDFs from a T1D background provide a vital model for studying the cellular and molecular mechanisms underlying impaired wound healing, a severe and common complication of diabetes. These cells may exhibit altered proliferation, migration, ECM remodeling, and inflammatory responses, making them essential for diabetes research, drug discovery, and the study of diabetic complications.

*Differentiation efficiency may vary due to the inherent biological variability of the obese donor phenotype.

These cells exhibit biological characteristics associated with the obese donor phenotype, which may impact their growth, differentiation, and metabolic behavior compared to cells from lean donors. It is the user's responsibility to determine the suitability of this product for their specific application.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

KEY FEATURES & BENEFITS

- **Disease-Relevant Model:** Sourced from verified Type 1 Diabetic donors, providing a physiologically relevant system to study diabetic dermopathy and impaired wound healing.
- **Key Cellular Player in Fibrosis & Repair:** Ideal for studying ECM deposition, contraction, and cell-ECM interactions in a diabetic context.
- **Characterized Phenotype:** Verified for expression of standard fibroblast markers (Vimentin, Fibronectin) and the absence of epithelial and endothelial contaminants.
- **Quality Controlled:** Every lot is tested for viability, sterility, and proliferative capacity to ensure experimental consistency.

APPLICATIONS

- **Diabetic Wound Healing Research:** Model impaired cell migration, proliferation, and ECM synthesis in vitro.
- **Fibrosis & Scarring Studies:** Investigate altered collagen deposition and contractile activity.
- **Drug Screening:** Test compounds aimed at enhancing fibroblast function and promoting healing in a diabetic context.

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- **Co-culture Systems:** Ideal for establishing models with keratinocytes (to study re-epithelialization) or immune cells to investigate inflammation.
- **Cellular Senescence & Aging:** Study accelerated aging and senescence pathways in diabetic complications.
- **ECM-Biology:** Analyze the composition and mechanical properties of the diabetic ECM.

QUALITY CONTROL SPECIFICATIONS

Each lot is tested to meet the following release criteria:

Parameter	Specification	Test Method
Viability (Post-Thaw)	≥ 80%	Trypan Blue Exclusion
Cell Yield per Vial	≥ 500,000 Cells	Cell Count
Morphology	Spindle-shaped, fibroblastic	Microscopic Evaluation
Immunofluorescence	Positive: Vimentin, Fibronectin Negative: Cytokeratin (Epithelial), CD31 (Endothelial)	IF Microscopy
Proliferation Assay	Positive for population doubling within specified timeframe	Functional Assay
Microbiological Sterility	No Growth	USP <71>
Mycoplasma	Negative	PCR or Culture Method

TYPICAL DONOR DEMOGRAPHICS & CLINICAL HISTORY

Donors are carefully selected and characterized. A typical profile includes:

- **Diagnosis:** Clinically confirmed Type 1 Diabetes Mellitus.
- **Age Range:** 18 - 65 years
- **Sex:** Male and Female donors available
- **Medication:** Insulin therapy
- **Tissue Source:** Redundant skin (e.g., from abdominoplasty) or foreskin.
- **Common Comorbidities:** May include other autoimmune conditions or early-stage microvascular complications.

HANDLING OF ARRIVING CELLS

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells into a -80°C freezer for short-term storage or liquid nitrogen tank for long-term storage.

3. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

4. When handling cell cultures, the application of stringent aseptic techniques is imperative. Maintaining a contamination-free environment is crucial for preserving the integrity and reliability of experimental results. This involves not only cleanliness but also the use of sterile tools, media, and working environments to prevent contamination from airborne particles, microbial agents, and other sources.

MEDIUM

We recommend Type 1 Diabetes Human Dermal Fibroblast Growth Medium (cat. FGM001) for these cells. The growth medium contains ingredients to support cell growth.

PROTOCOL FOR THAWING THE CELLS

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Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. Quick Thaw: Remove the vial from liquid nitrogen and immediately place in a 37°C water bath. Gently agitate until only a small ice crystal remains (≈1-2 minutes).

2. Decontaminate: Spray the vial with 70% ethanol before transferring to a biosafety cabinet.

3. Transfer and Dilute: Gently transfer the cell suspension to a 15 mL conical tube. Slowly add 5-9 mL of pre-warmed complete growth medium drop-wise to dilute the cryoprotectant (DMSO).

4. Centrifuge: Spin at 200 x g for 5 minutes.

5. Resuspend and Plate: Aspirate the supernatant and gently resuspend the cell pellet in fresh, pre-warmed complete growth medium. Plate cells into a culture flask pre-hydrated with 2-3 mL of medium.

6. Recommended Seeding Density: 5,000 - 10,000 cells/cm².

7. Incubate: Place the culture vessel in a humidified 37°C incubator with 5% CO_2.

8. Medium Change: Replace the medium after 24 hours to remove any non-adherent cells or debris, and then every 2-3 days thereafter.

SUBCULTURING PROCEDURE

1. Wash: Once cells are 70-90% confluent, aspirate the medium and rinse the cell layer gently with PBS.

2. Trypsinize: Add enough pre-warmed Trypsin/EDTA solution to cover the cell layer (e.g., 2 mL for a T-75 flask). Incubate at 37°C for 2-4 minutes. Observe under a microscope until cells round up and detach.

3. Neutralize: Add an equal volume of pre-warmed complete growth medium to neutralize the trypsin.

4. Centrifuge and Resuspend: Transfer the cell suspension to a tube and centrifuge at 200 x g for 5 minutes. Aspirate the supernatant and resuspend in fresh medium.

5. Re-plate: Seed cells into new culture vessels at a recommended split ratio of 1:3 to 1:4.

Note: Cells may exhibit slower proliferation rates compared to healthy donor HDFs. Adjust subculture schedules and seeding densities accordingly.

CRYOPRESERVATION

To bank cells, subculture as described and resuspend the pellet at 1-2 x 10^6 cells/mL in a cryopreservation medium (e.g., FBS with 10% DMSO). Aliquot into cryovials and freeze using a controlled-rate freezer, or place vials in an isopropanol freezing container at -80°C for 24 hours before transferring to liquid nitrogen for long-term storage.

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