NeoBioPharma
Advancing Health Through Innovation.

Data Sheet

Human Primary Diabetic Cardiac Microvascular Endothelial Cells

Catalog Number	NBPD003
Species & Source	Human. Isolated from the Cardiac tissue of donors diagnosed with Type II diabetes.
Cell Format	Cryopreserved cells delivered frozen in vials.
Cell Quantity	**≥500,000 viable cells per vial**.
Passage Number	**Passage 1**.
Quality Control - Sterility	Routinely tested negative for **mycoplasma, bacteria, yeast, and fungi**.
Quality Control - Pathogens	Tested and confirmed negative for **HIV-1, Hepatitis B (HBV), and Hepatitis C (HCV).**
Characterization Markers	**Positive for endothelial markers**: Positive for CD31 (PECAM-1) - Positive for von Willebrand Factor (vWF) / Factor VIII - Positive for VE-Cadherin - Positive for Acetylated LDL (DiI-Ac-LDL) uptake
Guaranteed Expansion	Guarantee expansion for a minimum of **15 population doublings**.
Shipping	Shipped on **dry ice** for overnight delivery.
Storage	Upon arrival, cells must be transferred immediately to **liquid nitrogen vapor phase (-180°C to -196°C)** for long-term storage. **Never** store at -20°C or a standard -80°C freezer.

A) Pre-Coating the T25 Flask:

Begin the process by thoroughly preparing the T25 flask to enhance cell adherence. Pour 2 ml of the NeoCoating Solution (NBP-01) into the center of the flask. Gently tilt and rotate the flask to ensure the solution spreads evenly across the entire surface, creating a homogeneous coating that promotes optimal cell attachment. Allow this coating to sit undisturbed for about 5 minutes, providing ample time for the solution to adhere properly to the flask's surface. After this incubation period, carefully aspirate any excess coating solution to avoid creating puddles that could interfere with cell growth. To further refine the attachment surface, rinse the flask thoroughly with 10 ml of 1x PBS, ensuring that all residual coating is removed. Once rinsed, the flask is now primed and ready for the vital process of cell seeding.

B) Thawing the Frozen Cell Vial:

With the T25 flask meticulously prepared, turn your attention to thawing the frozen cell vial. Submerge the vial in a 37°C water bath, taking care to keep the water level below the cap to maintain sterility and prevent contamination. Gently agitate the vial, allowing the warmth to thaw the contents, which should take just a few minutes. Once fully thawed, swiftly transfer the cells into the prepared T25 flask containing 10 ml of nutrient-rich NBP-02 medium, specifically designed to support optimal cell recovery and growth. Under ideal conditions, the cells will typically reach confluency overnight—an indication that they are ready for the next passage in their lifecycle.

C) Rinsing the Confluent Cells:

When the cells reach confluency, it becomes crucial to gently wash them to remove any lingering dead cells or debris that may have accumulated. Using a sterile technique, rinse the confluent cells in the T25 flask with 5 ml of Hanks' Balanced Salt Solution (HBSS) at room temperature. Repeat this rinsing process twice, ensuring comprehensive cleaning of the surface. Afterward, introduce 2 ml of Trypsin/EDTA (RT) (NBP-23) into the flask; this solution will initiate the delicate detachment of the cells from their substrate. It's essential to aspirate any excess Trypsin/EDTA within a tight timeframe of 20 seconds to prevent any detrimental effects from over-trypsinization that could compromise cell viability.

D) Cell Detachment Process:

Following the application of Trypsin/EDTA, let the T25 flask sit undisturbed at room temperature, or return it to the 37°C incubator for a minute. Keep a close eye on the cell detachment process, as most cells will begin to round up and detach within 1 to 2 minutes.

For a clearer view, employ a microscope to observe the cells' behavior; they will exhibit a rounded morphology as they release from the surface. If some stubborn cells remain attached, a gentle tap on the flask against the workbench can help dislodge them. Once a majority of the cells appear rounded, add 5 ml of Neutralization Buffer to halt the activity of trypsin. Carefully transfer the mixture into a centrifuge tube and centrifuge at 800 RPM for 5 minutes to pellet the cells effectively.

E) Resuspending the Cell Pellet:

After centrifugation, gently remove the supernatant without disturbing the delicate cell pellet that has formed at the bottom of the tube. Resuspend this cell pellet with care, using either 10 ml or 15 ml of NBP-02 medium, depending on the desired final volume for your experiments. For subculturing, transfer 5 ml of this resuspended cell solution into each of 2 or 3 newly pre-coated T25 flasks, achieving a precise 1:2 or 1:3 subculture ratio that supports continued growth and expansion.

F) Routine Medium Change:

To ensure a thriving cellular environment, it is essential to change the growth medium every 2 to 3 days. In the case of a 1:3 split ratio, the cells usually reach confluency again within about 7 days, signaling that they are well-prepared for further passages or can be utilized for experimental applications.

G) Preparing Quiescent Cells:

To prepare cells for specific assays while promoting a quiescent state, replace the NBP-02 medium with Endothelial Basal Medium (NBP-03) supplemented with 0.5% Fetal Bovine Serum (FBS) once the cells are nearing confluency. This transition is pivotal; incubate the cells within this medium for a duration of 8 to 12 hours before commencing any experimentation, as it aids in establishing a quiescent status that closely mimics physiological conditions, ultimately enhancing the relevance and reliability of your assay results.

By diligently following these detailed protocols, you will ensure optimal cell health and performance for your ongoing experiments.

Warranty: 1-month quality warranty period.

Note: Quality is not warranted if NeoBioPharma media is not used.

Caution: Human tissue–derived products may contain biological hazards. Even though each cell strain is screened and found negative for major pathogens such as HIV, HBV, and HCV, as well as for detectable DNA contaminants, no diagnostic test is perfectly reliable. As a result, there is always a residual risk of exposure to infectious agents. To avoid contamination, always wear gloves and safety glasses when working with these materials. Never mouth pipette. These precautions represent the minimum level of care required to reduce the risk of contamination or exposure when working with human tissue–derived products.