NEUROMICS

Immortalized Human Coronary Artery Endothelial Cells

Catalog #: HEC07-IM

Cell #: >5x10^5 cells

Storage: Liquid Nitrogen until ready for culture.
While culturing keep in 37°C CO_2 incubator (95% air, 5% CO_2)

Product Format: Frozen Vial

GENERAL INFORMATION

Endothelial cells were isolated from normal coronary artery tissue. Cells were then immortalized to allow for continued culture beyond the passage limits of typical primary cells. It is recommended to culture these cells following the protocols described below.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

STATEMENT

Handling human tissue derived products is potentially bio-hazardous, despite testing negative for HIV, HBV, and HCV DNA. Nonetheless, proper precautions must be taken to avoid inadvertent exposure.

SPECIAL NOTES:

- We strongly advise our customers to use medium and related products recommended by Neuromics, because our cells were grown and adapted using our products.
- The growth featured of our cells cannot be guaranteed if the specific growth mediums stated in our datasheets are not used.

UNPACKING AND STORAGE INSTRUCTIONS

- Check all containers for leakage or breakage. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.
- 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.

REQUIRED SUPPORTING REAGENTS

- ENDO-Growth Media (cat. MED001) or ENDO-Growth Media Kit (cat. EGK001)
- ENDO-Basal Media (cat. MED002)
- Cell Detachment Solution (cat. ADF001)
- AlphaBioCoat Solution (cat. AC001)
- Endothelial Cryopreserve Media (cat. FM300) *(if planning to freeze down cells)*

www.neuromics.com

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PROTOCOL

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.

Thawing Frozen Cells

1. Prepare Coated Flasks: Coat a T25 flask with Alphabiocoat (1 mL for 30 min at 37°C or overnight at 4°C), then aspirate excess before use.
2. Rinse: Using 1xPBS
3. Warm Medium: Pre-warm growth medium to 37°C.
4. Quick Thaw: Remove cryovial from liquid nitrogen and thaw in a 37°C water bath (~1–2 min).
5. Dilute Cells: Transfer cell suspension into a 15 mL tube and slowly add 5 mL of warm medium (dropwise to reduce osmotic shock).
6. Centrifuge: Spin at 200 × g for 5 min to pellet cells.
7. Resuspend & Plate: Aspirate supernatant, resuspend in fresh medium, and transfer to the coated flask.
8. Incubate: Place in a 37°C, 5% CO_2 incubator.

Routine Maintenance & Passaging

1. Monitor Growth: Check cells daily under a microscope (fibroblasts should have a cobblestone morphology at confluence and should be passaged at 80-90% confluence).
2. Change Medium: Replace medium every 2–3 days
3. Cell Detachment: Aspirate medium and wash cells gently with PBS. Add 1–2 mL of Cell Detachment Solution and incubate at 37°C for 2–3 min (check detachment under a microscope).
4. Neutralize: Add 2–3 mL of the neutralization solution to the T-25 flask
5. Collect Cells: Pipet gently to ensure single-cell suspension.
6. Centrifuge (Optional): If needed, spin at 200 × g for 5 min and resuspend in fresh medium.
7. Split Ratio: Seed cells at a 1:2 to 1:4 ratio.

Cryopreservation

1. Harvest Cells: Follow steps above to collect cells.
2. Count Cells: Use a hemocytometer to determine cell concentration.
3. Freezing: Resuspend cells in cryopreservation medium (~1–5 × 10^6 cells/mL).
4. Aliquot: Transfer 1 mL per cryovial.
5. Freeze Slowly: Use a freezing container (isopropanol-based) at –80°C overnight, then transfer to liquid nitrogen.

Quality Control & Troubleshooting

- Contamination Check: Regularly inspect for microbial contamination (cloudy medium, pH changes).
- Morphology: Endothelial cells should maintain a cobblestone monolayer at confluence. Elongation or spindle shapes suggest contamination of stress.
- Doubling Time: Monitor growth rates; immortalized lines should proliferate consistently.

Expected Results

- Healthy endothelial cells should adhere within 24h and proliferate with a doubling time of ~24–48h.
- Cells can typically be passaged 20–30 times before senescence

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