NEUROMICS

Immortalized Human Prostate Fibroblast Cells

Catalog #: HPF003-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

Human cells exhibiting fibroblast morphology were isolated from normal prostate 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

- Immortalized Prostate Fibroblast Growth Media (cat. FGM004)
- Cell Detachment Solution (cat. ADF001)
- AlphaBioCoat Solution (cat. AC001)
- Immortalized Fibroblast Cryopreservation Media (cat. FGM007) *(if planning to freeze down cells)*

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com

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 Media: Warm growth medium to 37°C.
2. Quick Thaw: Remove cryovial from liquid nitrogen and thaw in a 37°C water bath (~1–2 min). Remove from the water bath, spray with 70% alcohol
3. Transfer Cells: Pipet cell suspension into a 15 mL conical tube and slowly add 5–10 mL of pre-warmed medium (dropwise to minimize osmotic shock).
4. Centrifuge: Spin at 200 × g for 5 min to pellet cells.
5. Resuspend & Plate: Aspirate supernatant, resuspend in fresh medium, and transfer to a T25 flask coated with AlphaBioCoat Solution.
6. Incubate: Place in a 37°C, 5% CO_2 incubator.

Routine Maintenance & Passaging

1. Monitor Growth: Check cells daily under a microscope (fibroblasts should appear spindle-shaped and reach ~80–90% confluency before passaging).
2. Aspirate Medium: Remove spent medium and wash cells gently with PBS (1–2 mL for T25).
3. Cell Detachment: Add 1–2 mL of Cell Detachment Solution and incubate at 37°C for 2–5 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 up and down 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 (adjust based on growth rate).

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: Fibroblasts should maintain spindle-shaped morphology; if rounding occurs, check for over-confluence or stress.
- Doubling Time: Monitor growth rates; immortalized lines should proliferate consistently.

Expected Results

- Healthy fibroblasts 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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