NEUROMICS

Human iPSC

Catalog #: IPS013

Cell #: >1.0x10^6 cells

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

Product Format: Frozen Vial

GENERAL INFORMATION

The Human Induced Pluripotent Stem Cell (iPS) Line (Normal) is derived from dermal fibroblasts of a healthy adult donor. These cells have been reprogrammed using a non-integrating, virus-free method to express key pluripotency factors, reverting them to an embryonic stem cell-like state.

This cell line exhibits the defining characteristics of pluripotency, including the capacity for unlimited self-renewal and the ability to differentiate into derivatives of all three primary germ layers (ectoderm, mesoderm, and endoderm). It is an essential tool for disease modeling, drug discovery and toxicity screening, developmental biology research, and regenerative medicine.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

KEY FEATURES & BENEFITS

- **Reprogramming Method:** Non-integrating Sendai Virus Vectors (OCT4, SOX2, KLF4, c-MYC)
- **Parent Cell Type:** Dermal Fibroblasts (Normal, Healthy Donor)
- **Karyotype:** Normal, 46, XY (or 46, XX) - G-band resolution of 400-450. Confirmed at passage 7.
- **Mycoplasma Status:** Negative (Routine PCR testing)
- **Sterility:** Negative for bacterial and fungal contamination.
- **Pluripotency Status:** Verified by immunocytochemistry, flow cytometry, and trilineage differentiation.

APPLICATIONS

- **Human Disease Modeling:** Generate patient-specific disease models in vitro.
- **Drug Screening & Toxicology:** Test efficacy and safety of novel compounds on human cells.
- **Developmental Biology:** Study early human development and cell fate decisions.
- **Cell Therapy Research:** Differentiate into specific cell types (e.g., cardiomyocytes, neurons, hepatocytes) for regenerative medicine applications.
- **Gene Editing:** An ideal background for CRISPR/Cas9 studies due to normal karyotype.

QUALITY CONTROL SPECIFICATIONS

Each lot is tested to meet the following release criteria:

Pluripotency Marker Expression:

- Immunocytochemistry (ICC): Positive for OCT4, SOX2, NANOG, SSEA-4, TRA-1-60, TRA-1-81.
- Flow Cytometry: >95% of the cell population expresses OCT4 and NANOG.

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In Vitro Trilineage Differentiation Potential: The cell line successfully differentiates into cells of the three germ layers, confirmed by specific marker expression:

- Ectoderm: βIII-Tubulin (Neurons)
- Mesoderm: α-SMA (Smooth Muscle) / Brachyury
- Endoderm: SOX17 (Definitive Endoderm) / AFP

In Vivo Pluripotency:

- Capable of forming teratomas in immunocompromised mice, containing tissues representative of all three germ layers.

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 iPSC Maintenance Media, 500 ml (cat. IPSM001) for these cells. The medium contains ingredients to support cell growth.

PROTOCOL FOR CULTURING THE CELLS

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. Thaw vial quickly in a 37°C water bath.
2. Transfer cells to a tube with pre-warmed culture medium.
3. Centrifuge gently (200 x g for 5 minutes) to remove DMSO.
4. Resuspend pellet in fresh medium.
5. Plate onto an AlphaBioCoat Solution (cat. AC001) pre-coated culture vessel.
    a. Recommended Seeding Density: 10,000 - 20,000 cells/cm²
6. Once confluent, aspirate medium and wash with DPBS (without Ca^{2+}/Mg^{2+}).
7. Add appropriate dissociation reagent (Cell Detachment Solution – cat. ADF001 – is recommended) and incubate until colonies detach.
8. Neutralize with culture medium, collect cells, and centrifuge.
9. Resuspend in fresh medium and plate at the recommended density.
10. To cryopreserve, harvest cells as during passaging.

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11. Resuspend cell pellet in cold cryopreservation medium at a density of 1-2 x 10^6 cells/mL.
12. Aliquot into cryovials and freeze using a controlled-rate freezer.
13. Store vials in the vapor phase of liquid nitrogen

THAWING OF CELLS TIPS:

1. **Immediate Thawing Recommendation:** As soon as you receive frozen cell vials, it is imperative to thaw them and initiate cell culture swiftly. This quick action is vital for maximizing cell viability; any delay in thawing can critically compromise recovery rates and overall cell health, reducing the chances of successful cultivation.
2. **Preparation Coated Plates:** Before recovering the cells, meticulously prepare the AlphaBioCoat coated plates. This preparation is crucial as it sets up an optimal substrate that fosters robust cell growth and facilitates adhesion, particularly in feeder-free environments essential for the successful establishment of your cultures.
3. **Thawing the Cells:** To begin the thawing process, gently place the vial of frozen cells into a water bath maintained at precisely 37°C. Make sure to submerge only the bottom portion of the vial, avoiding water contact with the cap to prevent contamination. Agitate the vial lightly for 1 to 2 minutes. This careful, gradual warming is designed to ensure uniform thawing of the cells while minimizing the risk of thermal shock that could deleteriously affect cell viability.
4. **Transfer to Culture Medium:** Once the cells are fully thawed, transfer the entire contents of the vial into a sterile 15 mL conical tube pre-filled with 5 mL of complete iPS cell culture maintenance medium that has been pre-warmed to 37°C. This step is essential for promoting optimal recovery of the cells, ensuring they are in a favorable environment as soon as they are thawed.
5. **Centrifugation Process:** Subject the tube to a low-speed centrifugation at 50-100g for 5 minutes at room temperature. This gentle centrifugation effectively pellets the viable cells at the bottom of the tube while simultaneously removing the cryoprotectant that was necessary during the freezing process.
6. **Resuspension of Cell Pellet:** Once centrifugation is complete, take great care to aspirate the supernatant without disrupting the delicate cell pellet at the bottom. Gently resuspend the pellet in culture medium. Perform this procedure with caution to retain the cells in small clusters, as this characteristic is critical for their recovery and subsequent growth phase.
7. **Seeding the Cells:** Seed the gently resuspended cells onto the meticulously prepared AlphaBioCoat coated plates. It is recommended to distribute the cells at three different densities within 6-well plates. This strategy allows you to determine the optimal conditions for recovery and proliferation, enhancing the chance for a successful adaptation of the cells to their new culture environment.
8. **Incubation:** Place the seeded plates in a 37°C CO2 incubator, providing a stable thermal environment conducive to cell growth. Allow the cells to incubate overnight, which enables them to settle and begin adhering firmly, further enhancing their chances for successful recovery.
9. **Ongoing Media Changes:** Conduct daily changes of the culture medium until the cells reach the appropriate confluency for passaging. It is important to note that the full recovery process can take anywhere from 1 to 2 weeks, varying by the specific lot number of the cells. During this time, you may observe approximately 5-10% of the cells differentiating. To maintain culture purity, manually remove these differentiated cells using a sterile tip or syringe under a microscope while working within a sterile biosafety cabinet, which helps ensure a contaminant-free environment. Expect the cell population to stabilize following 2-3 passages.

SUBCULTURING TIPS

1. **Preparation Pre-coated Plates:** Begin the subculture process by preparing pre-coated plates meticulously per AlphaBioCoat Datasheet.

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2. **Washing Cells:** Upon reaching 80-90% confluence—which suggests that the cells are nearing full growth but remain in a healthy state—initiate the washing procedure. Gently aspirate the existing culture medium from each well, exercising care to avoid disturbing the delicate monolayer of cells. Rinse the cells with 2 mL of sterile phosphate-buffered saline (PBS) per well, ensuring complete removal of any residual medium, metabolic waste, or dead cells. This critical washing step is vital for maintaining cellular health and prepares the cells for subculturing, enhancing their viability and reducing contamination risks.

3. **Cell Dissociation:** Following the wash, add 1 mL of Cell Detachment Solution to each well. Distribute the reagent evenly across the surface of the cells to ensure comprehensive coverage. Allow the cells to incubate at room temperature for 1-2 minutes to permit the reagent to penetrate the cell layers and initiate the dissociation process. After this initial exposure, carefully aspirate the Cell Detachment Solution. Place the plate in a 37°C incubator for an additional 3-4 minutes to optimize the dissociation effect. Note: Depending on the specific characteristics of the cell line you are working with, you may need to adjust the dissociation time; some cell lines may require longer exposure to achieve effective detachment while maintaining high cell viability.

4. **Detaching Cell Colonies:** After the incubation period, introduce 1 mL of Neutralization Media to each well. This step serves to neutralize the Cell Detachment Solution and halt the dissociation process. Gently tap the sides of the plate using a sterile pipette or similar object to dislodge the colonies without applying excessive force, which could disrupt the integrity of the aggregates. It's important to avoid using pipettes at this stage, as too much suction can lead to unwanted separation of aggregates into individual cells, compromising their viability.

5. **Transferring Cell Aggregates:** Once the colonies have been successfully detached, carefully transfer the cell aggregates into a 15 mL conical tube containing 5 mL of fresh culture medium. When performing this transfer, utilize a wide-bore pipette tip or a sterile spatula, if necessary, to minimize shear stress on the aggregates. This is important to preserve the structural integrity of the cell clusters and promote optimal recovery during the subsequent culture period.

6. **Centrifugation of Cell Aggregates:** Centrifuge the conical tube at a relative force of 50-100g for precisely 5 minutes at room temperature. This gentle centrifugation causes the cell aggregates to form a pellet at the bottom of the tube while minimizing potential cellular damage. After centrifugation, cautiously resuspend the pellet in the desired volume of fresh culture medium. Take care to gently swirl or flick the tube to achieve resuspension of the aggregates without fragmenting them into single cells, ensuring they remain intact for further plating.

7. **Plating the Cells**: Plate the resuspended cell aggregate mixture onto pre-coated 6-well plates at the density recommended for your specific cell line. Utilize the specified medium, supplemented with 10 µM Y-27632, to enhance cell viability and prevent apoptosis during this critical adaptation phase. This ROCK inhibitor supports the survival of cells under stress conditions inherent during subculturing. After a 24-hour acclimatization period in this medium, transition the cells to standard culture media that does not contain Y-27632, allowing the cells to fully adapt to their new environment and promoting optimal growth conditions.

8. **Monitoring Cell Growth and Confluence:** If the cells are seeded at the optimal density, anticipate that they will progress toward confluence and be ready for experimental application within 4 to 7 days. Regularly monitor the cultures for consistent growth patterns, cellular morphology, and overall confluence. Be prepared to change the culture medium as needed to support robust cellular health and functionality. Regular observations will help identify any signs of overgrowth or differentiation that may require your attention.

By meticulously following these detailed steps, you can ensure a successful and efficient subculture process that optimizes cell line maintenance, promoting their readiness for subsequent experimental applications.

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