NEUROMICS

Human IPSC – Cortical Neurons

Catalog #: IPS002

Cell #: >5x10^5 cells

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

Product Format: Frozen Vial

Expected Viability: 55%

Volume: 1 ml

GENERAL INFORMATION

Human IPSC-Cortical Neurons are derived from integration-free induced pluripotent stem cell (iPSC) lines under a fully defined proprietary neural induction condition. The source of the cells are primary fibroblasts, which were obtained from a healthy donor. This cell line provides a unique model system for better understanding of iPSC-derived cortical neurons. Cells are provided at passage 1.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

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 at a temperature below-130°C, preferably in liquid nitrogen vapor, until ready for use.

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.

PRODUCT TESTING

- Negative for bacteria, yeast, fungi, and mycoplasma

MEDIUM

We recommend customers use our IPSC Neuron Plating Medium (cat. HNM003) and Neuron Growth Media (cat. HNM001) to culture these cells.

PROTOCOL FOR THAWING 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.

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1. Prepare the coated vessel before thawing cells. IPSC-cortical neurons have been tested for quality control (QC) using AlphaBioCoated precoated plates.

2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1-2 minutes. Keep the cap out of water to minimize the risk of contamination.

3. Remove the vial from the water bath as soon as the contents are thawed. Decontaminate by dipping in or spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.

4. Pipette the cells into a 15 mL conical tube with 5 mL neuron plating medium.

5. Centrifuge at 500g for 5 minutes at room temperature.

6. Remove the supernatant and re-suspend the cells in plating medium. Cells should be resuspended in plating medium to minimize cell death. Plating should be removed after 24 hours by replenishing fresh medium carefully.

7. Seed the cells on an AlphaBioCoat (cat. AC001) coated culturing surface at the desired density (information on coating can be found in the subculture protocol below). Add 6.0 to 8.0 mL of Neuron Growth Medium (cat. HNM001). Incubate in 37°C CO2 incubator overnight.

   Note: We recommend seeding 100-200K cells per cm2 depending on the application. Cell debris may be observed after cell recovery because the cryopreserved neurons are fragile. Refer to the CoA of each lot to determine the seeding density for your experiment.

   Note: AlphaBioCoat precoated flasks, well plates, and dishes are also available for purchase.

8. Perform half medium change every 2-3 days with growth medium. Most of the cells should express high levels of TUJ1 and MAP2 2-3 days after thaw, and express synaptic markers such as SV2 and PSD-95 seven days after thaw.

   Note: Pure cortical neurons tend to aggregate and detach from the plates. Change 50% of the medium with extra care to avoid cell loss.

SUBCULTURING PROCEDURE

Note: Volumes are given for a 75 cm2 flask. Increase or decrease the amount of medium needed proportionally for culture vessels of other sizes.

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1x PBS, remove and discard 1x PBS.
3. Add 2.0 to 3.0 mL of Cell Detachment solution (cat. ADF001) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

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4. Add 6.0 to 8.0 mL of AlphaBioCoat solution to the T-Flask for 15 minutes. Aspirate the solution after 15 minutes, rinse with 8ml of 1XPBS. Discard the 1XPBS.
5. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
6. To remove cell detachment solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 37°C.

CAUTION

Handling human tissue-derived products is potentially bio-hazardous. Although each cell strain is tested negative for HIV, HBV, and HCV DNA, diagnostic tests are not necessarily 100% accurate; therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.

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