



## Human Neuron Precursor Cells

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**Catalog #:** HNPC001

**Cell #:** >5x10<sup>5</sup> cells

**Storage:** Liquid Nitrogen until ready for culture.  
While Culturing keep in 37°C CO<sub>2</sub> incubator

**Product Format:** Frozen Vial

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### GENERAL INFORMATION

Human Neuron Precursor Cells (HNPC) are offered in frozen vial at passage 2. These cells have a minimum average population doubling Capacity > 6 when cultured following the detailed protocol described below.

*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
- Negative for HIV-1, HBV, and HCV

### MEDIUM

We recommend customers use our Quick Coating Solution (cat. AC003) and Human Neuron Precursor Cells Growth Medium (cat. HNM012) 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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### FOR RESEARCH USE ONLY

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1. Pre-coat T25 flasks with Quick Coating Solution 1-2 hours before thawing the cells. To do this, add 2ml of solution into one T25 flask and make sure whole surface of the flask is covered with the solution. Five minutes later, dispose excess solution by aspiration and the flask is ready to be used (no need for overnight incubation when using Quick Coating Solution). Another extracellular matrix can be used including gelatin, collagen, and fibronectin.
2. Thaw the cells and place the vial in a 37°C water bath with gentle agitation for 1-2 minutes. It's important to keep the cap out of the water to minimize the risk of contamination. Spray the vial with 70% ethanol, wipe the vial, and place it under your biosafety cabinet.
3. Pipette the cells into a 15 mL conical tube with 5 mL of Human Neuron Precursor Cells Growth Medium. Centrifuge at 200g for 5 minutes at room temperature.
4. After removing the supernatant, re-suspend the cells in 5 ml of Human Neuron Precursor Cells Growth Medium and transfer into a pre-coated T25 flask.
5. Incubate the cells in a 37°C CO2 incubator overnight.
6. Change medium every 2-3 days

### **SUBCULTURE PROTOCOL**

1. Passage the cells when they reach 80-90% confluency.
2. Prepare plates coated with Quick Coating Solution 1-2 hours before splitting the cells.
3. Remove the media from the cells and rinse with 5 ml HBSS (Room Temperature, RT) twice.
4. Add 2ml of Trypsin/EDTA (RT) into the T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution within 60 seconds with aspiration
5. Leave the T25 flask with the cells at 37C for extra 1-2 minute (the cells usually will detach from the surface within 1-2 minutes). You can monitor the cells under microscope and when most of cells become rounded up, hit the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.
6. Add 5 ml Trypsin Neutralization Buffer (cat. NS002) and spin the cells down with 800g for 5 minutes.
7. Re-suspend the cell pellet with 10-15 ml of Human Neuron Precursor Cells Growth Medium and the cell suspension is transferred directly into 2 or 3 pre-coated T25 flasks (5 ml each, and the cells are sub-cultured at 1:2 to 1:3 ratio).
8. Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:3 ratio).

### **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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