# **Human PSC-Derived Dopaminergic Neurons**



Cryopreserved human PSC-derived dopaminergic neuron precursor cells for maturation into midbrain-type dopaminergic neurons

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Catalog #70909 1 million cells XCL-1, Male

# **Product Description**

These cryopreserved human pluripotent stem cell (PSC)-derived dopaminergic neuron precursor cells will generate a population of tyrosine hydroxylase (TH)-expressing dopaminergic neurons (> 30% TH-positive dopaminergic neurons,  $\geq$  80% class III  $\beta$ -tubulin-positive neurons; < 15% GFAP-positive astrocytes) that are functional and can be maintained long-term in culture. Each vial contains  $\geq$  1 x 10^6 viable precursor cells that can be fully differentiated and matured using STEMdiff<sup>TM</sup> Dopaminergic Neuron Maturation Kit (Catalog #08530). These cryopreserved neural cells are convenient, highly consistent, and allow rapid implementation of physiologically relevant human PSC-based models for drug discovery, cell therapy validation, and neuroscientific research.

# Stability and Storage

Stable at -135°C or colder for 2.5 years from date of manufacture (MFG) on label.

Short-term storage of cells (< 2 weeks) at -80°C is acceptable, but should be minimized to ensure maximum stability. Upon receipt, immediately transfer vials from dry ice to storage units, avoiding exposure to room temperature. Thawed samples must be used immediately.

## **Precautions**

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

# Materials Required But Not Included

| PRODUCT NAME   | CATALOG #   |
|--|-------------|
| STEMdiff <sup>™</sup> Dopaminergic Neuron Maturation Kit | 08530       |
| Poly-L-ornithine hydrobromide (PLO) Solution             | Sigma P4957 |
| Laminin  | Sigma L2020 |
| Falcon® Conical Tubes, 15 mL                             | 38009       |
| Trypan Blue  | 07050       |

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## Directions for Use

For medium and cultureware preparation instructions (e.g. poly-L-ornithine/laminin coating), refer to the Product Information Sheet (PIS) for STEMdiff<sup>TM</sup> Dopaminergic Neuron Maturation Kit (Document #DX20343), available at www.stemcell.com or contact us to request a copy.

#### THAWING CRYOPRESERVED CELLS

NOTE: Pre-warm cultureware and media that will come in contact with cryopreserved cells. This protocol is for a single vial (1 mL) of cryopreserved cells. If using other volumes, adjust accordingly. 1 mL of cryopreserved cells can be cultured on a 60 mm cell culture dish or a 6-well plate.

- 1. Warm 2 x 5 mL aliquots of STEMdiff™ Dopaminergic Neuron Maturation Medium 1 at 37°C in 15 mL conical tubes.
- 2. Place warm tubes of medium and warm cultureware in a biosafety cabinet.
- 3. Remove the vial of cryopreserved cells from liquid nitrogen storage and immerse up to 2/3 of vial height in a 37°C water bath.
- 4. Remove vial from water bath when only a small piece of ice is visible; this will take approximately 1 minute for 1 mL of cells. Do not shake vial during thawing process.
- 5. Wipe the outside of the vial with 70% ethanol or isopropanol and immediately place inside the biosafety cabinet containing the pre-warmed medium.
- 6. Use a 1 mL pipettor to slowly transfer thawed cell suspension into one of the 15 mL conical tubes containing warm medium. Carefully transfer cell suspension dropwise into medium while swirling. Do not pipette cells up and down or generate bubbles, as cells are in a very fragile state.
- 7. Centrifuge diluted cell suspension at 300 x g for 5 minutes at room temperature (15 25°C).
- 8. Aspirate supernatant carefully, leaving behind a small amount of medium. Take care not to disturb the cell pellet.
- 9. From the second tube of warm medium, remove 1 mL and add to the cell pellet. **Gently resuspend** the cells by pipetting up and down slowly 4 6 times.
- 10. Perform a cell count using Trypan Blue (10 μL cell suspension + 10 μL Trypan Blue) and a hemocytometer.
- 11. Dilute cell suspension with an appropriate volume of the remaining STEMdiff™ Dopaminergic Neuron Maturation Medium 1 in order to achieve the required seeding density. Seed cells onto poly-L-ornithine/laminin-coated cultureware and culture as described in section C of Directions For Use in the PIS for STEMdiff™ Dopaminergic Neuron Maturation Kit (Document #DX20343), available at www.stemcell.com or contact us to request a copy.

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