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Product Information

Dopaminergic Neuron Culturing Information for iPSC-differentiated neurons

Catalog Numbers **DOPA1001, DOPA1002, DOPA1003, DOPA1014, DOPA1028, DOPA1037, and DOPA1038**
Storage Temperature –196 °C (Liquid Nitrogen, LN_2)

TECHNICAL BULLETIN

Product Description

These products contain cryo-preserved, pre-differentiated dopaminergic neuron precursors derived from a footprint-free, karyotype normal human iPSC line. They are designed for customers to generate mature dopaminergic neurons using the optimized maturation medium and supplements. Mature and functional dopaminergic neurons can be obtained within 12–14 days. The dopaminergic precursors can be seeded on various culture vessel formats including 96 well plates on either glass or plastic surfaces, and cultured as adherent cells. Shortly after seeding, the cells proliferate slightly for up to 4 days and show extensive neurite outgrowth and proper neuronal morphology. In general, at 12–14 days post-seeding, the cell population will contain ≥80% neurons, ≥30% TH positive dopaminergic neurons, and ≤ 15% Glial Fibrillary Acidic Protein (GFAP) positive astrocytes.

Reagents and Equipment Required but Not Provided

Dopaminergic Medium with Supplement A and Supplement B
- Catalog Number DOPA1050 [50 mL of medium]
- Catalog Number DOPA1051 [250 mL of medium]

Poly-L-ornithine hydrobromide, Catalog Number P3655

Mouse Laminin solution, Life Technologies Catalog Number 23017-015

0.4% Trypan Blue solution, Catalog Number T8154

Primary antibodies:

Monoclonal anti-β-tubulin III, isotype III clone SDL.3D10, Catalog Number T8660

Polyclonal Rabbit anti-TH (Anti-Tyrosine Hydroxylase) Antibody, Pel-Freez Biologicals, Catalog Number P40101

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The storage conditions for the dopaminergic neuron precursors and the required Dopaminergic Medium are shown in Table 1.

A vial with cryopreserved dopaminergic neuron precursors is packed in a small Ziploc® bag, which is buried in the dry ice. Upon receiving the product, check the integrity of the package and the presence of dry ice.

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from the dry ice during transportation to liquid nitrogen storage units. Transfer the cryopreserved dopaminergic neuron precursors directly to liquid nitrogen storage units from the dry ice, avoiding exposure to room temperature.

Re-freezing the Dopaminergic Medium and supplements, or cryopreserving cultured dopaminergic neurons is not recommended.

Table 1.
Storage conditions of dopaminergic neuron precursor components

Component	Storage Temperature
Cell vials	–196 °C (Liquid Nitrogen, LN_2)
Dopaminergic Medium	–20 °C
Supplement A	–20 °C
Supplement B	–20 °C

Dopaminergic Neuron Maturation Procedure
This procedure has been extensively tested with the dopaminergic neuron precursors and Dopaminergic Medium. The user should follow this procedure closely. The user assumes all responsibility for the failure of the experiment should there be any deviation from this procedure.

Coating Cell Culture Vessels with Poly-L-ornithine and Laminin
Cell culture vessels should be coated one day before or on the day of plating the cells.

1. Prepare a stock solution of poly-L-ornithine (10 mg/mL) by dissolving the powder in sterile cell culture grade water. The stock solution should be stored at –20 °C.
2. Thaw a 1 mg bottle of Mouse Laminin solution (Life Technologies Catalog Number 23017-015) on ice.
3. Prepare working solution of poly-L-ornithine in sterile cell culture grade water to a final concentration of 20 μg/mL.
4. Add poly-L-ornithine solution into desired cell culture vessel to entirely cover the growth surface of the vessel (see Table 2).
5. Distribute the solution evenly and incubate vessels in a cell culture incubator for 2 hours (37 °C/ 5% CO_2 with humidity control).
6. Rinse vessels twice with cell culture grade water. Pipette water gently toward the corner of the vessel to avoid mechanical removal of poly-L-ornithine coating.
7. Dilute the Mouse Laminin solution in sterile cell culture grade water to a final working concentration of 10 μg/mL.
8. Aspirate water from the vessels and add laminin working solution to entirely cover the bottom of the vessel. Incubate in the cell culture incubator for 2 hours (37 °C/5% CO_2 with humidity control).
9. It is recommended to use freshly coated vessels. However, if not used immediately, store coated vessels at 2–8 °C in laminin solution (up to 4 days).
10. Pre-warm vessels at 37 °C before use.
11. Aspirate laminin just before seeding dopaminergic neuron precursors. Do not let the surface dry.

Table 2.
Recommended volumes of coating reagents for various vessels

Vessel type	poly-L-ornithine	Laminin
96 well plate	50 μL/well	50 μL/well
4 or 24 well plate	250 μL/well	250 μL/well
35 mm dish	1.5 mL	1.5 mL
60 mm dish	2.5 mL	2.5 mL

Thawing and Culturing Cryopreserved Dopaminergic Neuron Precursors
All steps described must be performed in accordance with aseptic cell culture standards. All media and vessels used for cell culture must be pre-warmed to 37 °C prior to use.

For the entire process of dopaminergic neuron maturation, two types of complete media are required:

- Complete Medium A (Dopaminergic Medium with Supplement A) is used for culture from day 0-5
- Complete Medium B (Dopaminergic Medium with Supplement B) is used for culture from day 5–12, and up to day 35.

1. One day before thawing precursor cells, store the Dopaminergic Medium at 2–8 °C overnight. Once thawed, medium can be stored at 2–8 °C for up to 3 weeks.

2. On the day of thawing precursor cells, transfer 20 mL aliquot of Dopaminergic Medium into 50 mL conical tube and add the entire contents (80 μL) of Supplement A (pre-thawed on ice) to make Complete Medium A.

3. Add a 5 mL aliquot of Complete Medium A to a 15 mL conical tube and pre-warm at 37 °C. This aliquot will be used for recovery of precursor cells from frozen stock.

4. Prepare another aliquot of Complete Medium A according to volumes required for cell culture vessels utilized (see Table 3). Pre-warm at 37 °C only enough Complete Medium A for use in cell culture that day. Store the remaining Complete Medium A at 2–8 °C.

Table 3.
Recommended volumes of medium in various vessels

Vessel type	Medium volume
96 well plate	100 μL/well
4 or 24 well plate	500 μL/well
35 mm dish	2 mL
60 mm dish	5 mL

5. Shortly before thawing the cells, place pre-warmed medium and vessels in the biosafety cabinet.

6. To thaw dopaminergic neuron precursors, remove one vial from liquid nitrogen and place immediately onto dry ice (the vial must be buried in the dry ice).

7. Bring dry ice container with the vial to a 37 °C water bath and immerse the vial in the bath (up to 2/3rd of the vial) and thaw cells until only a small piece of ice is still visible (~1 minute).
   Note: Do not shake vial during thawing.

8. Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol solution and wiping with an autoclaved paper towel.

9. Remove cells from the vial using a micropipette (or serological pipette) and transfer drop-wise while swirling into 15 mL conical tube containing 5 mL of pre-warmed Complete Medium A (step 3). Wash the vial with 1 mL of medium from the 15 mL conical tube and transfer it back to the tube.
   Note: Do not shake vial during thawing.

10. Centrifuge cells at 400 × *g* for 5 minutes at room temperature.

11. Aspirate the medium very carefully using a vacuum (or pipette if preferred) leaving only a drop of liquid in the tube. Do not remove or disturb the cell pellet during aspiration of medium.

12. Using a micropipette, add 1 mL of Complete Medium A (step 4) into the tube and gently re-suspend cells by pipetting up and down 4–6 times.

13. Remove a 10 μL aliquot of cell suspension and mix it with 10 μL of 0.4% Trypan blue solution.

14. Count the cells.

15. Calculate appropriate volume of Complete Medium A needed according to the vessel used (see Table 3). Re-suspend cells in Complete Medium A and seed them at density ranging from 4×10^4 to 6×10^4 live cells/cm^2.

16. Distribute cells evenly and place vessels in the cell culture incubator (37 °C/5% CO_2 with humidity control). Medium should be changed every other day.

17. Monitor cell survival the following day.

18. Change Complete Medium A at **day 2** post-seeding. Medium change should be done slowly (drop wise) pointing the pipette tip toward the wall of cell culture vessel.

19. At **day 5** post-seeding, make Complete Medium B by transferring 15 mL of Dopaminergic Medium into a 50 mL tube and add the entire contents (30 μL) of one vial of Supplement B (pre-thawed on ice).

20. Take an aliquot of Complete Medium B according to which cell culture vessels are being utilized (see Table 3). Pre-warm the aliquot and place the rest of Complete Medium B at 2–8 °C.
    Note: Pre-warm only as much Complete Medium B as needed. Keep the remaining medium at 2–8 °C.

21. Replace Complete Medium A with Complete Medium B.

22. Prepare more Complete Medium B when needed, as described in step 20.

23. Continue differentiation of dopaminergic neurons in Complete Medium B. Change medium every other day. Differentiation can be terminated at **day 12–14** post seeding for downstream applications.
    Note: Cells can be differentiated for up to 5 weeks post-seeding. However, prolonged culture will have increased population of GFAP positive astrocytes.

Results

Characterization of Mature Dopaminergic Neurons

The maturation of dopaminergic neurons can be assessed by their morphology, see Figure 1, and by immunostaining using three markers, see Figure 2. Percentage of dopaminergic neurons can be determined by TH (Tyrosine Hydroxylase) positive neurons divided by the total number of cells (DAPI staining of nuclei).

Figure 1.
Example of dopaminergic neuron morphology at different time points post-seeding.

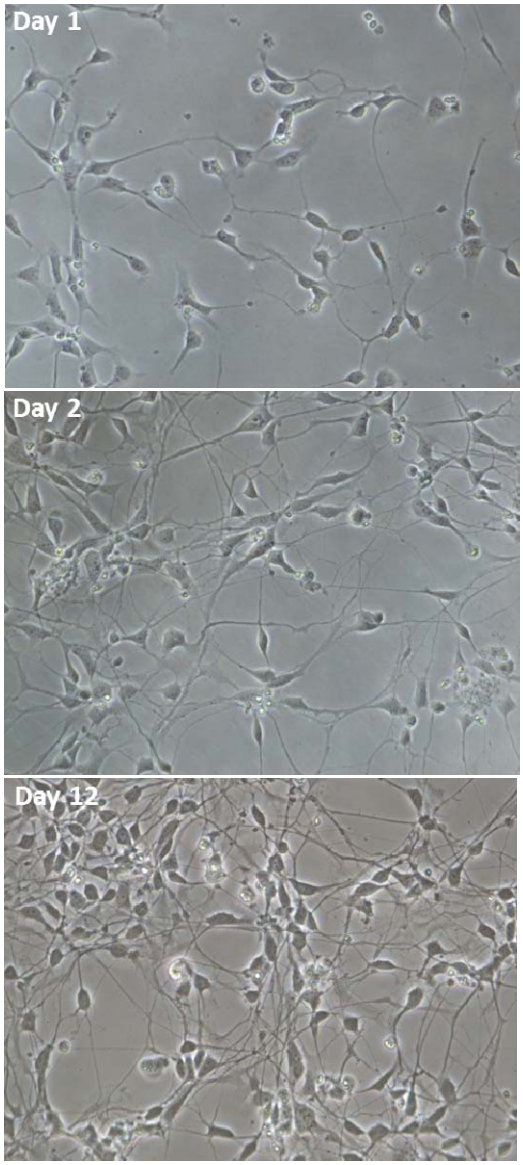

Figure 2.
Example of mature dopaminergic neurons. Immunostaining at 12 days post-seeding, showing ≥85% of total cells expressed Tuj-1 marker (green) and ≥30% expressed TH marker (red). Total count of nuclei (blue) is used as the total number of cells.