Dopaminergic Neurons Starter Kit (iPSC from Blood Cells; Male)

Product Information

Catalog Number **ASE-9323K**

Description

Human Dopaminergic Neuron Kit contains cryo-preserved, pre-differentiated dopaminergic neuron precursors derived from a footprint free, karyotype normal human iPSC line. It is designed for customers to generate mature dopaminergic neurons using optimized maturation medium andsupplements. 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, on Day 12-14 post-seeding, the cell population will contain >80% neurons, >30% TH+ dopaminergic neurons and <15% GFAP+ astrocytes.

Characterization of Dopaminergic Neurons at Maturity

Maturation of dopaminergic neurons can be assessed by their morphology and by immunostaining using three markers (see example below). Percentage of dopaminergic neurons can be determined by TH (Tyrosine Hydroxylase) positive neurons divided by the total number of cells (DAPI staining of nuclei).

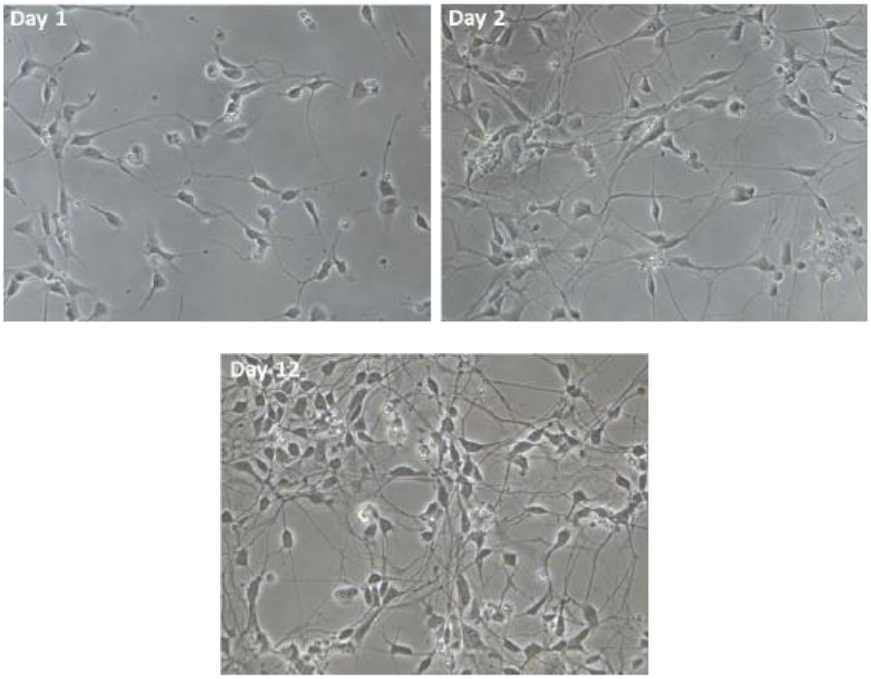

Figure 1. Example of DOPA cell morphology at different time points post-seeding.

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

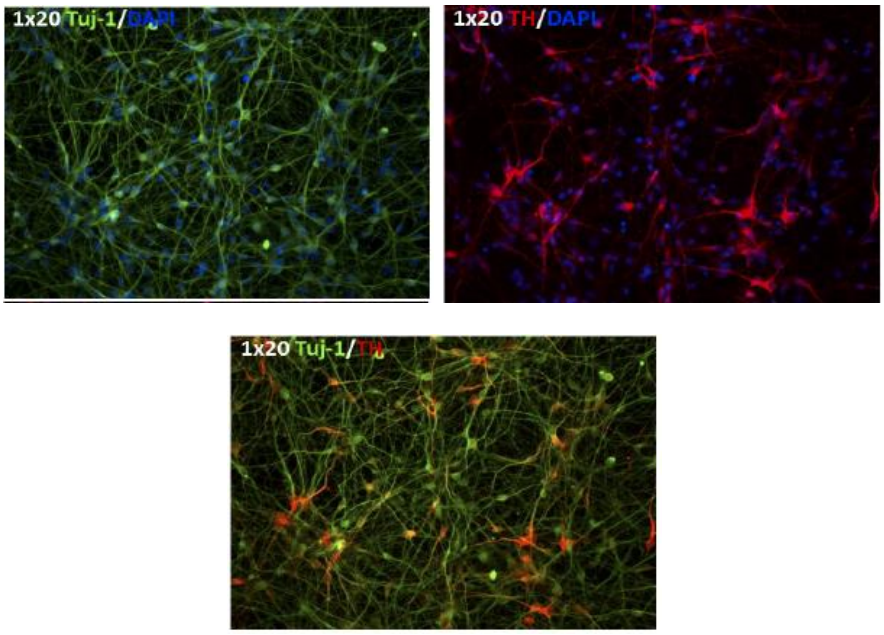

Figure 2. Example of mature dopaminergic neurons. Immunostaining on Day 12 post-seeding, showeing that >85% of total cells expressed Tuj-1 marker (green) and >30% expressed TH marker (red). Total count of nuclei (blue) is ued as the total number of cells. .

Shipping

Dry ice at ambient temperature

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Warranty

Performance of DOPA precursors has been extensively tested withother components of the DOPA Starter Kit. amsbio will not hold responsibilityif components other than components of the DOPA Starter Kit are used to culture dopaminergic precursors provided with the kit.

Media and Material

DOPA Starter Kit (ASE-9323K)

Component	Amount	Storage	Shelf Life
DOPA Precursors	1 vial ≥1 x 10^6 viable cells	Liquid Nitrogen	12 months
DOPA Medium	1 x 50 mL	-20°C	12 months
Supplement A	1 vial	-20°C	12 months
Supplement B	1 vial	-20°C	12 months

Additional Reagents

- Recommended:
  - Poly-L-ornithine hydrobromide, Sigma, Cat# P3655
  - Laminin, Life Technologies, Cat# 23017-015

- Primary antibodies:
  - Mouse anti-β III-tubulin isotype III clone SDL.3D10, Sigma, Cat# T8660
  - Polyclonal Rabbit anti-TH (Anti-Tyrosine Hydroxylase) antibody, Pel-Freez Biologicals, Cat# P40101-150

Protocol

Notes:

- *We do not recommend re-freezing supplements and medium provided with the DOPA Starter Kit.*
- *We do not recommend cryopreserving DOPA precursors.*

1. Handling Upon Receiving

DOPA Starter Kit is shipped on dry ice. The components are packed in zip-lock bags. A small transparent bag with dopaminergic precursors is buried in dry ice, which is buried in dry ice. Upon receiving the product, check the integration of the packages and the presence of dry ice (contact amsbio if the integrity of thepackage has been compromised, e.g. no dry ice in the package)

Cells can be plated immediately after arrival or transferred into deep freezer (-80°C) for short term storage (up to 2 weeks) or into liquid nitrogen for long term storage (up to 6 months). Do not remove the vials from dry ice during transportation to storage units. Immediately transfer DOPA Starter Kit components (especially DOPA precursors) to storage units, avoiding exposure to room temperature.

After arrival, properly store the kit's components as follows:

Component	Storage
DOPA Precursors	Liquid Nitrogen
DOPA Medium	-20°C
Supplement A	-20°C
Supplement B	-20°C

2. Procedure

This procedure has been extensively tested with DOPA Precursors and DOPA 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.

2.1 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.
Please read producer's manual for handling of Poly-L-ornithine and Laminin.

2.1.1. Prepare 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.

2.1.2. Thaw stock solution of laminin (1 mg/mL, Life Technologies) on ice.

2.1.3. Prepare working solution of poly-L-ornithine in sterile cell culture grade water at a final concentration of 20 µg/mL.

2.1.4. Add poly-L-ornithine solution into desired cell culture vessel to cover the vessel's bottom entirely (see Table 1).
2.1.5. Distribute the solution evenly and incubate vessels in the cell culture incubator for 2 hr (37°C/ 5% CO_2/ humidity control).
2.1.6. Rinse vessels two times with cell culture grade water. Pipette water gently toward the corner of the vessel to avoid mechanical removal of poly-L-ornithine coating.
2.1.7. Prepare working solution of laminin in sterile cell culture grade water at a final concentration of 10 µg/mL.
2.1.8. Aspirate water from the vessels and add laminin solution to cover well the vessel's bottom. Incubate in the cell culture incubator (37°C/ 5%CO_2/ humidity control) for 2 hours.
2.1.9. If not used immediately, store coated vessels at 4°C in laminin solution (up to 4 days).
2.1.10. Pre-warm vessels at 37°C before use.
2.1.11. Aspirate laminin just before seeding DOPA precursors. Do not let the surface dry.

Table 1. Recommended volumes of coating reagents in various vessels.

Vessel Type	P-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

2.2 Thawing and Culturing Cryopreserved DOPA Precursors

All steps described here 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: Medium A (DOPA Medium + Supplement A) is used for culture from **Day 0-4**; whereas Medium B (DOPA Medium + Supplement B) is used for culture from **Day 4-12** and up to **Day 35**.

2.2.1 One day before thawing precursor cells, place the 50 mL DOPA Medium bottle in 2°- 8°C fridge overnight.

Note: *Once thawed, DOPA MEDIUM can be stored at 2°- 8°C for up to 3 weeks.*

2.2.2 On the day of thawing precursor cells, transfer 20 mL aliquot of DOPA Medium into 50 mL conical tube and add the entire contents (80 µL) of Supplement A (pre-thawed on ice) to make the complete Medium A.
2.2.3 Take 5 mL aliquot of Medium A in a 15 mL conical tube and pre-warm at 37°C. This aliquot will be used for recovery of precursor cells from frozen stock.
2.2.4 Prepare another aliquot of Medium A according to volumes required for cell culture vessels utilized (see Table 2). Only take enough Medium A to be utilized for cell culture that day and pre-warm at 37°C. Place the rest of Medium A back to 2°- 8°C fridge.

Table 2. Recommended volumes of medium in various vessels

Vessel Type	Medium Volume
96 well plate	100 µL/well
4 or 24 well plate	50 µL/well
35 mm dish	2 mL
60 mm dish	5 mL

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

2.2.6 To thaw DOPA precursors, remove one vial from storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).

2.2.7 Bring dry ice container with the vial to the site with the 37°C water bath.

2.2.8 Immerse the vial in the water bath (up to 2/3 of the vial) and thaw cells until only a small piece of ice is still visible (approximately 1 minute).

Note: *Do not shake the vial during thawing.*

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

2.2.10 Remove cells from the vial using a p1000 micropipette (or serological pipette) and transfer drop-wise while swirling into a 15 mL conical tube containing 5 mL of pre-warmed Medium A (step 3). Wash the vial with 1 mL of solution from the 15 mL conical tube and transfer it back to the tube.

Note: *Do not mix cells up and down and avoid generation of bubbles.*

2.2.11 Centrifuge cells at 400 x g for 5 minutes at room temperature.

2.2.12 Aspirate the medium carefully using vacuum (or pipette if preferred).

2.2.13 Using p1000 micropipette add 1 mL of Medium A (step 4) into the tube and gently resuspend cells by pipetting up and down 4-6 times.

2.2.14 Remove 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.

2.2.15 Count the cells.

2.2.16 Calculate appropriate volume of Medium A needed according to the vessel used (See Table 2). Resuspend cells in Medium A and seed them at density ranging from 4×10^4 to 6×10^4 live cells/cm^2. See Table 3 for your convenience.

Table 3. Recommended seeding densities for neurons in various types of cell culture vessels. Range: low to high.

Vessel	Surface/Well	Medium Volume	Density (Cells)
96-well plate	0.33 cm^2	100 µL/well	$1.3 \times 10^4 – 1.9 \times 10^4$
4-well plate	2 cm^2	500 µL/well	$8 \times 10^4 – 1.2 \times 10^5$
35 mm dish	10 cm^2	2 mL	$4 \times 10^5 – 6 \times 10^5$
60 mm dish	20 cm^2	5 mL	$8 \times 10^5 – 1.2 \times 10^6$

2.2.17 Distribute cells evenly and place vessels in the cell culture incubator (37°C/ 5% CO_2/humidity control). This is **Day 0**; Medium should be changed ever other day.

2.2.18 Monitor the cells' survival and attachment the following day (**Day 1**).

2.2.19 Change Medium A on **Day 2.** Medium change should be done slowly (drop wise), pointing the pipette tip toward the wall of cell culture vessel.

2.2.20 On **Day 4**, make Medium B by transferring 15 mL of DOPA Medium into a 50 mL tube and add half of the contents (30 µL) from the vial of Supplement B (pre-thawed on ice).

2.2.21 Take an aliquot of Medium B according to cell culture vessels utilized (See Table 2). Pre-warm the aliquot and place the rest of Medium B at 2°- 8°C.

Note: *Pre-warm only as much medium as is needed. Keep the remaining medium at 4°C.*

2.2.22 Replace Medium A with Medium B.

2.2.23 Prepare more Medium B when needed, as described in Step 20.

2.2.24 Continue differentiation of dopaminergic neurons in Medium B. Change medium every other day. Differentiation can be terminated on **Day 12-14** for downstream applications.

Note: *Cells can be differentiated for up to 5 weeks. However, prolonged culture will have increased population of GFAP positive astrocytes.*

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85