Dopaminergic Neurons
(iPSC from Blood Cells; Female)

Product Information

Catalog Number	**ASE-9323F**
Description	Human Dopaminergic neurons are cryo-preserved, pre-differentiated dopaminergic neuron precursors derived from a footprint-free, karyotypenormal human iPSC line (cord blood-derived female iPSC line). These precursors yield high purity mature dopaminergic neurons using optimized dopaminergic maturation medium and supplements (DOPA maturation media; ASE-9323DM). The dopaminergic precursors can be seeded on various culture vesselformats 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. Mature dopaminergic neurons can be obtained within 12 days after thaw, and the matured dopaminergic neurons can be maintained in culture for up to 3 weeks without losing functionality.
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).
Quality Control	≥90% Tuj-1 positive (Neuronal class III β-Tubulin) ≥30% TH (Tyrosine Hydroxylase) ≤5% GFAP positive
Recovery of Frozen Cells	≥80% viability
Amount	≥1x10^6 viable cells/vial
Shipping	Dry-ice
Storage	Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed
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.

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Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.
Warranty	Performance of dopaminergic neurons has been extensively tested with other components. AMSBIO will not hold responsibility if media other than the recommended maturation media is used.

Additional Reagents Required for Dopaminergic neuron maturation

- DOPA Maturation Medium, AMSBIO, Cat# ASE-9323DM
- Poly-L-ornithine hydrobromide, Sigma-Aldrich, Cat# P3655
- Laminin, Life Technologies, Cat# 23017-015
- Cell culture grade water, Corning Cellgro, Cat# 25-055-CVC
- Mouse anti-β III-tubulin clone SDL.3D10, Sigma, Cat# T8660
- Polyclonal Rabbit anti-TH (Anti-Tyrosine Hydroxylase) antibody, Pel-Freez Biologicals, Cat# P40101-150

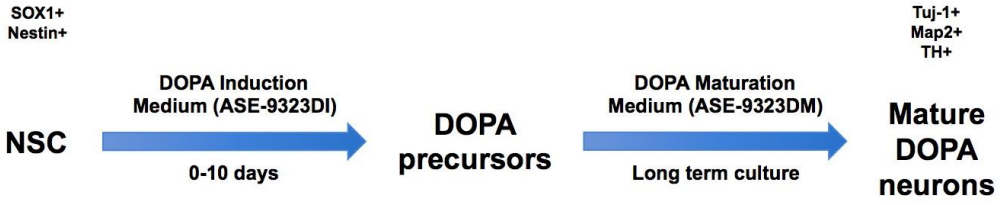
Note: *Equivalent products from other vendors can be used; however, we highly recommend having a small-scale pre-qualification done before switch.*

Equipment required for astrocyte differentiation

Equipment	Specifications
Vertical laminar flow hood	
Incubator	Maintain 37°C and 95% humidity in an atmosphere of 5% CO2
Low speed centrifuge	(e.g. Thermo Centra CLS)
Pipette-aid	
Serological pipettes	
Hemacytometer or Automated Cell Counter	(e.g. BioRad TC 10)
Inverted microscope	(e.g. Nikon TS100)
Appropriate culture-ware	Tissue-treated plates (e.g. 430167, 430145, 430196, corning)

Protocol

Process of dopaminergic (DOPA) differentiation using DOPA Induction (ASE-9323DI) and Maturation Media (ASE-9323DM)



1. Handling Upon Receiving

Dopaminergic neuron precursor cells are shipped on dry ice. Upon receiving the product, check the integration of the packages and the presence of dry ice (contact AMSBIO if the integrity of the package has been compromised, e.g. no dry ice in the package)

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from dry ice during transportation to storage units. Immediately transfer components (especially the cryopreserved precursor cells) to storage units, avoiding exposure to room temperature where required.

2. Coating Cell Culture Vessels with Poly-L-ornithine/Laminin

Cell culture vessels should be coated one day before or on the day of plating the cells.

2.1 Coating

2.1.1 Prepare a stock solution of poly-L-ornithine (10mg/mL) by dissolving the powder in sterile cell culture grade water. The stock solution should be aliquoted as desired and stored at -20°C.
2.1.2 Thaw a stock solution of poly-L-ornithine on ice.
2.1.3 Prepare working solution of poly-L-ornithine in sterile cell culture grade water (final concentration 20 µg/mL). To make sufficient volume for the experiment, please refer to Table 1.
2.1.4 Add poly-L-ornithine solution into desired cell culture vessel to cover growth surface entirely (see Table 1).
2.1.5 Distribute the solution evenly and incubate vessels in a cell culture incubator for 2 hours (37°C/ 5% CO_2/ humidity control).
2.1.6 In the meantime, thaw a stock solution of laminin (1 mg/mL) on ice.
2.1.7 Prepare a working solution of laminin in sterile cell culture grade water (f.c. 10 µg/mL). To make sufficient volume for the experiment, please refer to Table 1.
2.1.8 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.
2.1.9 Aspirate water from the vessels and add laminin solution to cover growth surface entirely. Incubate in the cell culture incubator (37°C/ 5% CO_2/ humidity control) for 2 hours.
2.1.10 We recommend that freshly coated vessels be used. However, if not used immediately, store coated vessels at 4°C in laminin solution (up to 4 days).
2.1.11 Pre-warm vessels at 37°C before use.
2.1.12 Aspirate laminin just before seeding cells. Do not let the surface dry. There is no need to wash vessels after removal of laminin.

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

Vessel	Approx. Surface Area	poly-L-ornithine	Laminin
96 well plate	0.33 cm^2/well	50 µL/well	50 µL/well
4 or 24 well plate	2 cm^2/well	250 µL/well	250 µL/well
35 mm dish	10 cm^2	1.5 mL	1.5 mL
60 mm dish	20 cm^2	2.5 mL	2.5 mL

3. Maturation of DOPA Precursors into Functional DOPA Neurons

During the DOPA neuron maturation phase, cell divisions will gradually decrease and DOPA precursors will elongate significantly, generate neuronal processes and mature.

All operations should 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.

3.1 Preparation of complete DOPA Maturation Media (ASE-9323DM)

Thaw media components overnight at 2°- 8°C. Complete medium shall be stored at 2°- 8°C and used within three weeks. Pre-warm an aliquot of complete medium at 37°C before use. Please note that there are two formulations for DOPA maturation: the first one consists of DOPA Maturation Basal Medium + Supplement A (complete formulation 1), to be used for **day 0-4** culture; and the second one consists of DOPA Maturation Basal Medium + Supplement B (complete formulation 2), to be used after **day 4** (Table 2).

Table 2. Formulation of complete DOPA Maturation Medium (100 mL)

Component	Storage	Volume Provided	Formulation Per 50 mL		Optional one-time re-freezing
			Day 0-4	Day 4 & up	
DOPA Maturation Basal Medium	Long term: -20°C Short term: 4°- 8°C (3 weeks)	1x100 mL	20 mL	30 mL	yes
DOPA Maturation Supplement A	-20°C	2x80 µL	80 µL		yes
DOPA Maturation Supplement B	-20°C	4x30 µL		60 µL	yes

3.2 Thawing and Culturing 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**.

3.2.1 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.*

3.2.2 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.

3.2.3 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.

3.2.4 Prepare another aliquot of Medium A according to volumes required for cell culture vessels utilized (see Table 3). 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.

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

3.2.6 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).

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

3.2.8 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.*

3.2.9 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and

wiping with an autoclaved paper towel.

3.2.10 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 (Point 3.2.3). 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.*

3.2.11 Centrifuge cells at 400 x g for 5 minutes at room temperature.
3.2.12 Aspirate the medium carefully using vacuum (or pipette if preferred).
3.2.13 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.
3.2.14 Remove 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
3.2.15 Count the cells.
3.2.16 Calculate appropriate volume of Medium A needed according to the vessel used (See Table 3). Resuspend cells in Medium A and seed them at density ranging from 4×10^4 to 6×10^4 live cells/cm^2.

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	$8 \times 10^5 – 1.2 \times 10^6$

3.2.17 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.
3.2.18 Monitor the cells' survival and attachment the following day (**Day 1**).
3.2.19 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.
3.2.20 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).
3.2.21 Take an aliquot of Medium B according to cell culture vessels utilized (See Table 3). 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.*

3.2.22 Replace Medium A with Medium B.
3.2.23 Prepare more Medium B when needed (as described in Step 3.2.20).
3.2.24 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.*

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