Applied StemCell, Inc.
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Datasheet

DOPA Maturation Media

Product Information

Catalog Number

ASE-9323DM

Description

DOPA Maturation Media is serum-free media produced using Applied StemCell's proprietary formulations to allow researchers to differentiate neural stem cells (NSC) into functional dopaminergic neurons (DOPA). Applied StemCell's induction and maturation media have been tested and optimized using NSC and neurons derived from Applied StemCell's control iPSC line, ASE-9109. The DOPA neuron differentiation process is divided into two stages: In the first stage, the DOPA Induction Medium (ASE-9323DI) is used to differentiate NSC into DOPA precursors. In the second stage, the DOPA Maturation Medium is used to further differentiate the precursors into high purity, mature, functional DOPA neurons and to maintain these neurons in long-term culture (up to 40 days) (See Figure 1). DOPA Induction Medium can be purchased separately (ASE-9323DI) or as part of the Dopaminergic Neurons Starter Kit (ASE-9323K/ ASE-9323KF).

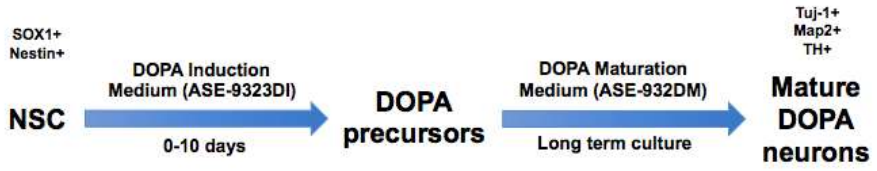

Figure 1. The process of dopaminergic differentiation using DOPA Induction and Maturation Media.

Quantity

DOPA Maturation Basal Medium: 100 mL; DOPA Maturation Supplement A: 160 µL; DOPA Maturation Supplement B: 120 µL.

Shipping

Dry ice

Storage and Stability

DOPA Maturation Basal Medium: Store at 2-8°C (short-term) and -20°C (long-term); DOPA Maturation Supplement A: Store at -20°C; DOPA Maturation Supplement B: Store at -20°C.

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.

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Warranty

The performance of DOPA maturation media has been tested extensively and optimized using NSC and DOPA neurons derived at Applied StemCell. However, the performance of these media may vary depending on the quality of the NSCs from different sources. Applied StemCell will not be held responsible if components other than those recommended with the kit are used for cell culturing.

Characterization of Dopaminergic Neurons at Maturity

Morphology

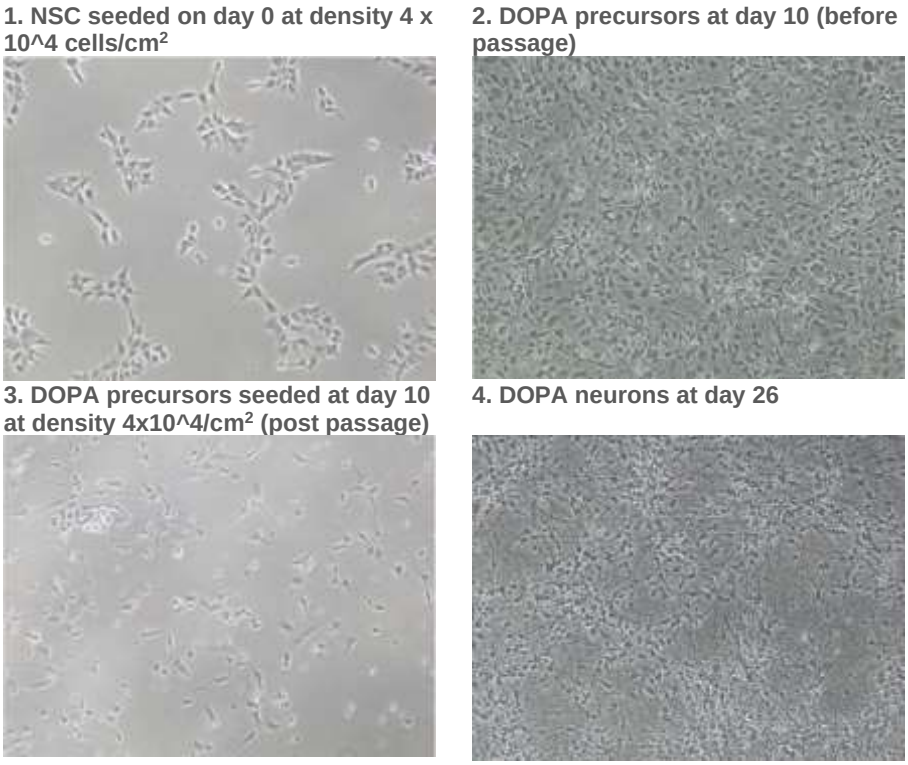
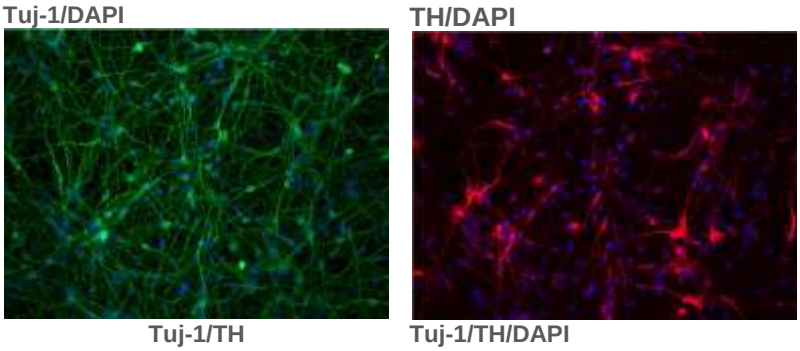


Figure 2. Example of neural stem cells (NSC), DOPA precursors, and DOPA neurons morphology: at different stages of differentiation using DOPA Induction (ASE-9323DI) and DOPA Maturation Media (ASE-9323DM).

Immunostaining



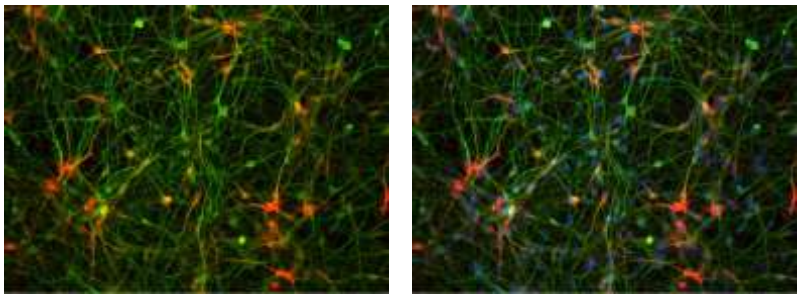


Figure 3. Immunostaining of dopaminergic (DOPA) neurons at day 22 of differentiation. More than 80% cell stain positive for Tuj-1 (neuronal class III β-tubulin marker; green) and >30% for TH (tyrosine hydroxylase; red). DAPI (blue) was used for staining the nucleus .

Media and Material

Components included with DOPA Maturation Media (ASE-9323DM):

Catalog#	Component	Amount	Storage	Shelf Life
ASE-9323DM-c	DOPA Maturation Medium	1 x 100 mL	-20°C	12 months
ASE-9323DM-a	Supplement A	1 x 160 µL	-20°C	12 months
ASE-9323DM-b	Supplement B	1 x 120 µL	-20°C	12 months

Additional Reagents

- Recommended:
  - Poly-L-ornithine hydrobromide, Sigma, Cat# P3655
  - Laminin, ThermoFisher, Cat# 23017015

- 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 Applied StemCell 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 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 x 10^4 to 6 x 10^4 live cells/cm^2. See Table 3 for your convenience.

Table 3. Recommended seeding densities for neurons in 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 x 10^4 – 1.9 x 10^4

4-well plate	2 cm^2	500 µL/well	8 x 10^4 – 1.2 x 10^5
35 mm dish	10 cm^2	2 mL	4 x 10^5 – 6 x 10^5
60 mm dish	20 cm^2	5 mL	8 x 10^5 – 1.2 x 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.*

Troubleshooting Guide

Problem	Possible causes	Suggestion
Cells reached confluence before day specified in the protocol	NSC lines derived using different protocols, may have different dynamics of proliferation and differentiation	Decrease seeding density. Adjust protocol by introducing additional cell passage step, if necessary.
Cells didn't survive passaging	Harsh treatment upon passaging; Increased time of incubation in accutase; Too vigorous mixing/washing of cells during passage	Do not keep cells in accutase solution longer than 5 min. Dilute accutase by adding pre-warmed DMEM into the plate with harvested cells before washing/mixing the cells.
Cell clumps visible after seeding onto new cell culture vessels	Too high density promotes cell clumping; Not efficient washing/mixing performed upon harvesting	In cases when cell clumps/granulates are visible by eye, please use cell strainer (minimum 40µM) to isolate clumps from single cells.
Neurons are peeling off in 96 well plates	This may randomly occur when the entire medium in each well is removed and replaced with new one. Full removal of medium generates forces that will peel neurons from the center of the well.	Replace 50% of the medium. Pipet slowly against walls of the wells to avoid mechanical peeling.