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 extensively tested and optimized using NSC and neurons derived from a variety of human pluripotent cells (hESC and hiPSC). The DOPA neuron differentiation process is divided into two stages. In the first stage, the DOPA Induction Medium is used to differentiate NSC into DOPA precursors. In the second stage, the DOPA Maturation Medium is used to further differentiate the precursors into 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-9323/ ASE-9323F).

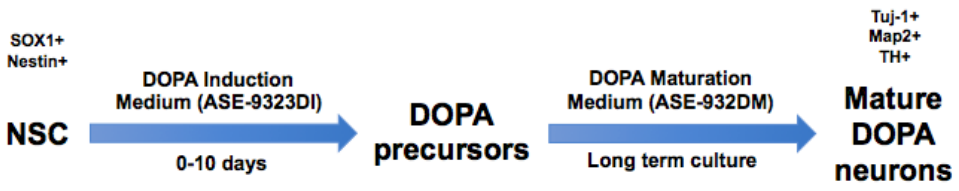

Figure 1. The process of dopaminergic differentiation using DOPA Induction and Maturation Media.

Quantity

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

Shipping

The components of the DOPA Maturation Media are shipped as two packages: the -20°C components are shipped on dry ice and the 2-8°C components are shipped in cooler containing cold inserts.

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. 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 Applied StemCell's DOPA Maturation Media has been extensively tested with other components. Applied StemCell will not hold responsibility if components other than the recommended components are used.

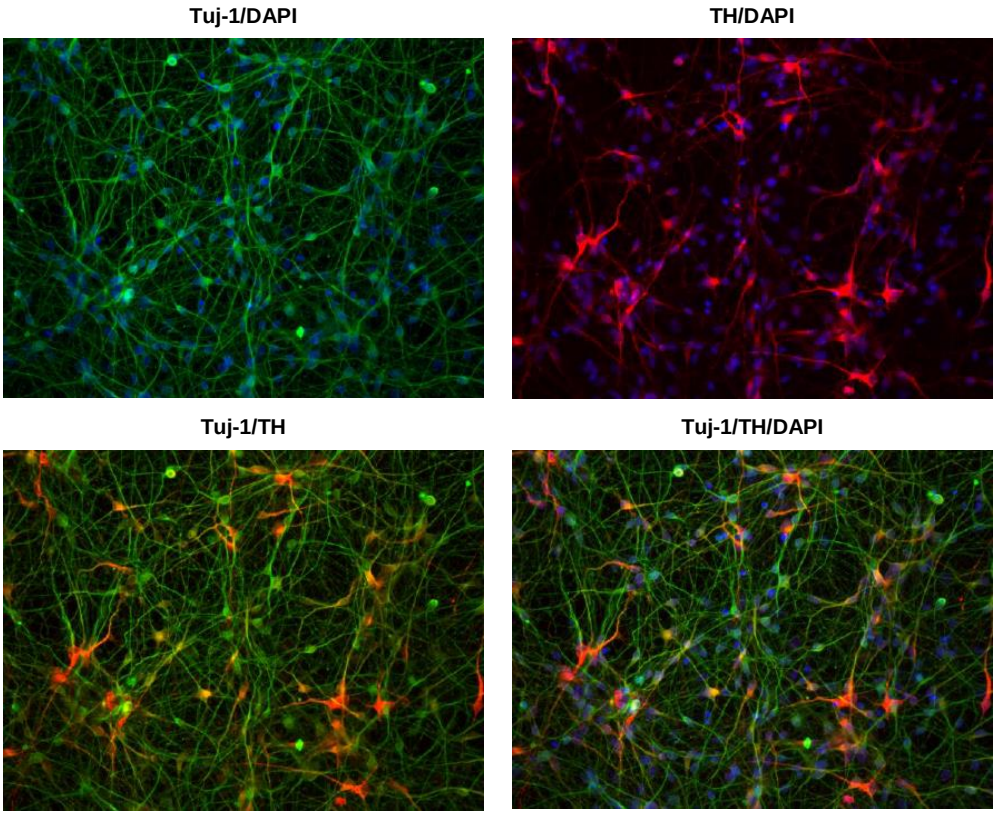

Figure 2. Immunostaining of DOPA neurons at day 22 of differentiation (day 22 induction phase; day 12 maturation phase). Tuj-1 (Neuronal Class III β-Tubulin) – green, TH (Tyrosine Hydroxylase) – red, Nuclei (DAPI) - blue

Media and Material

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

Cat. Number	Component	Amount	Storage
ASE-9323DM	DOPA Maturation Basal Medium	100 mL	Short-term: 2-8°C; Long-term: -20°C
ASE-9323DM-a	DOPA Maturation Supplement A	2x80 µL	-20°C
ASE-9323DM-b	DOPA Maturation Supplement B	4x30 µL	-20°C

Additional Reagents Required but not Provided

- Poly-L-ornithine hydrobromide, Sigma-Aldrich, Cat# P3655
- Laminin, Life Technologies, Cat# 23017-015
- Cell culture grade water, Corning Cellgro, Cat# 25-055-CVC
- Accutase (cell dissociation reagent), Life Technologies, Cat# A11105-01

- DMEM, Life Technologies, Cat# 12491-015

Protocol

1. Handling Upon Receiving

Components of Applied StemCell's DOPA Maturation Media are shipped as two separate packages: The -20°C components are shipped on dry ice; whereas the 2-8°C components are shipped in cooler containing cold inserts (8-15°C). Upon receiving the product, check the integrity of the packages and the presence of dry ice (contact Applied StemCell if the integrity of a package has been compromised, e.g. no dry ice in the package). Store components as specified.

2. Preparation of Culture Vessels and Media

The handling procedures described below have been extensively tested for all of Applied StemCell's NSC line (ASE-9234 and ASE-9234F) using specified substrate coating and Applied StemCell's optimized maintenance media. The user should follow these procedures closely. The user assumes all responsibility for the failure of the experiment should there be any deviation from these procedures.

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 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 (f.c. 20 µg/mL). To make sufficient volume for this 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 the 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 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 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

3. DOPA Maturation

The DOPA differentiation process is comprised of two phases. I) During the induction phase, differentiation of NSC into DOPA precursors will be initiated, which will be hallmarked by morphological changes in NSC (cell polarization and elongation), however the cells will still proliferate. II) During the maturation phase, cell divisions will gradually decrease and neural precursors will elongate significantly, generate neuronal processes and mature.

All steps described below were optimized using NSC produced by Applied StemCell. If NSC from other sources are used, the protocols might need further optimization according to performance of these cells. 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:

Use sterile techniques when preparing reagents and materials. Thaw media components overnight at 2°-8°C. Complete medium shall be stored at 2°-8°C and used within 3 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. Formulation of Complete DOPA Maturation Medium (e.g. 100 mL size)

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: 2°-8°C	1 x 100 mL	50 mL	30 mL	yes
DOPA Maturation Supplement A	-20°C	2 x 80 µL	80 µL		yes
DOPA Maturation Supplement B	-20°C	4 x 30 µL		60 µL	yes

3.2 DOPA Maturation

3.2.1 Aspirate Laminin solution from pre-warmed cell culture vessel and seed DOPA precursors at a density ranging from $4x10^4$ to $6x10^4$ live cells /cm^2 in DOPA Maturation Medium formulated for **days 0-4** (Figure 3). Suggested volumes are listed in Table 3.

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

3.2.2 Distribute cells evenly and place vessels in the cell culture incubator (37°C/ 5%CO_2/ humidity control).
3.2.3 Change medium every alternate day. To avoid cell peeling, media change should be done slowly (drop-wise) pointing the pipette tip toward the wall of cell culture vessel.
3.2.4 Continue maturation of DOPA neurons for a minimum of 14 days. DOPA neurons can be cultured for up to 5 weeks if prolonged maturation time is required.

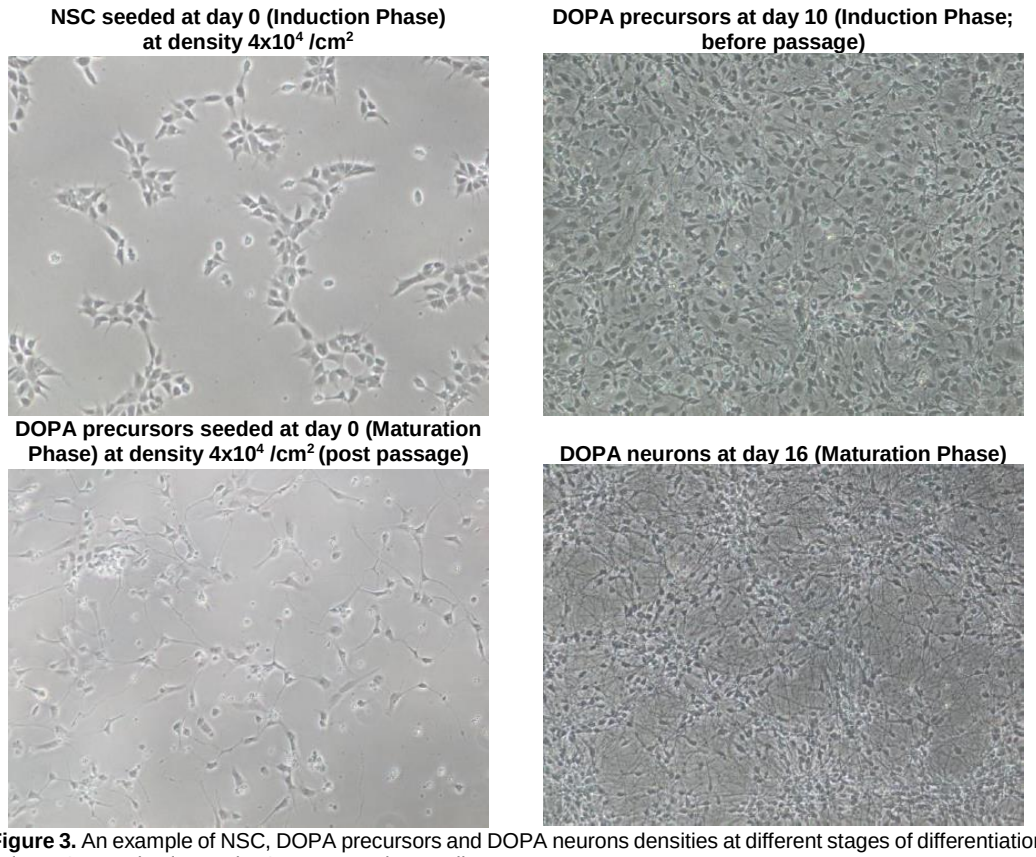

Figure 3. An example of NSC, DOPA precursors and DOPA neurons densities at different stages of differentiation using DOPA Induction and DOPA Maturation Media

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.