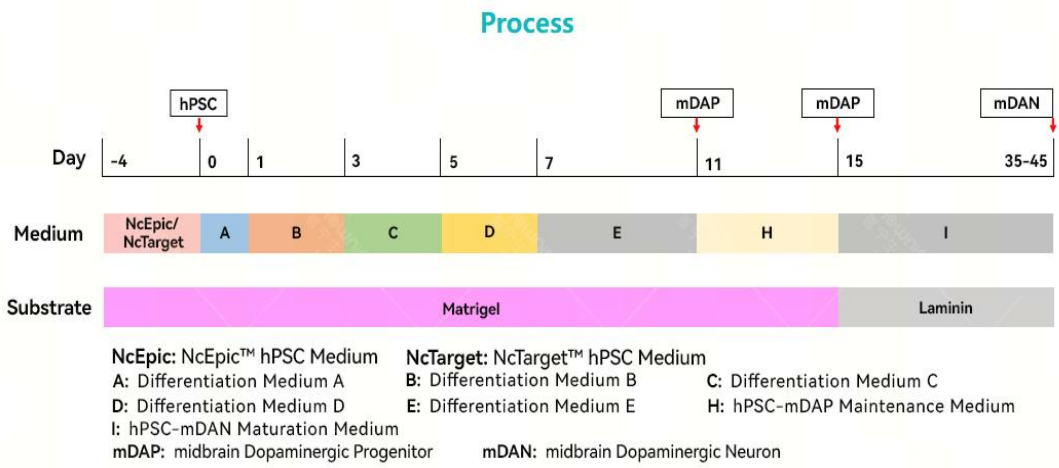
hPSC-mDAP Differentiation Kit
Product Manual

Cat.No.RP01017 1 Kit

I. Product Introduction

1.1. Product Description

The hPSC–mDAP Differentiation Kit is intended for the differentiation of human pluripotent stem cells (hPSCs) into midbrain dopaminergic progenitors (mDAPs) and their subsequent progression toward mature midbrain dopaminergic neurons (mDANs). The kit includes a hPSC-mDAP Differentiation Kit, a hPSC-mDAP Maintenance Medium, and a hPSC-mDAN Maturation Medium, which may be selected and combined flexibly according to specific experimental requirements. Using the hPSC-mDAP Differentiation Kit, highly enriched mDAPs (>90% Lmx1a$^+$/ Foxa2$^+$/ En1$^+$) can be obtained from hPSCs. The hPSC-mDAN Maturation Medium further supports the progression of mDAPs into mature mDANs (TH$^+$/Nurr1$^+$). The resulting human dopaminergic neural progenitors and mature dopaminergic neurons are suitable for applications in neurodegenerative disease research, drug screening, and the establishment of Parkinson's disease-related experimental models.



1.2. Product Information

Table 1: Product Components of the hPSC-mDAP Differentiation Kit

Production	Cat.No.	Amount	Storage
hPSC-mDAP Differentiation Kit*	RP01017	1 Kit	Basal medium: 2–8 °C Supplements:-20 °C or -80 °C
hPSC-mDAP Maintenance Medium	RP01017-H	100 mL	Basal medium: 2–8 °C Supplements:-20 °C to -80 °C
hPSC-mDAN Maturation Medium	RP01017-I	50 mL	

***Each kit enables the generation of more than 1 × 10^7 mDAPs (Day 11).**

***The complete differentiation medium, prepared by mixing the basal medium with supplements, can be stored at 2–8 °C for up to 2 weeks.**

1.3. Reagents and Materials

Table 2: Recommended Reagents and Materials

Reagents & Materials	Brand (e.g.)	Cat.No. (e.g.)
NcEpic™ hPSC Medium	Shownin	SN-01-0010
NcTarget™ hPSC Medium	Shownin	RP01020
Vitronectin	Shownin	RP01002
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin	Shownin	RP01008
hPSC Cryopreservation Medium	Shownin	SN-06-1210
Solase	Shownin	RP01021
ncLaminin511	Shownin	RP01025
TrypLE	Gibco	12604013
Corning® Matrigel® Matrix	Corning	354277
DMEM/F-12 Medium	Thermo Sci.	11330
DPBS, no calcium, no magnesium	Thermo Sci.	14190144
6-well plate	Thermo Sci.	140685
1 mL/5 mL/10 mL/25 mL Pipette	Thermo Sci.	N/A
15 mL/50 mL Centrifuge tube	Thermo Sci.	N/A
1.5/2 mL Cryopreservation tube	Thermo Sci.	N/A
1.5 mL Microcentrifuge tube	Axygene	N/A
10 µL/200 µL/1000 µL tip	Rainin .	N/A
Programmed freezing container	Thermo Sci.	5100-0001

II. hPSC-hDAP differentiation

2.1. Reagent Preparation

Table 3: Product Instruction of hPSC–mDAP Differentiation Kit

Product	Cat.No.	Amount	Storage
hPSC–mDAP Differentiation Kit* contains:	**RP01017**	**1 Kit**	
mDAP Differentiation Supplement A（100×）	RP01017-A	100 µL	-20 °C or -80 °C
mDAP Differentiation Supplement B（100×）	RP01017-B	200 µL	
mDAP Differentiation Supplement C（100×）	RP01017-C	200 µL	
mDAP Differentiation Supplement D（100×）	RP01017-D	200 µL	
mDAP Differentiation Supplement E（100×）	RP01017-E	600 µL	
mDAP Differentiation Basal Medium F	RP01017-F	120 mL	2–8 °C
mDAP Cryopreservation Medium G	RP01017-G	50 mL	

***Each kit enables the generation of more than 1 × 10^7 mDAPs (Day 11).**

***Each kit is sufficient for differentiation in 8 wells of a 12-well plate or 4 wells of a 6-well plate.**

***The complete differentiation medium, prepared by mixing the basal medium with supplements, can be stored at 2–8 °C for up to 2 weeks.**

2.1.1. Allow mDAP Differentiation Supplement A,B,C,D,E and Differentiation Basal Medium F to thaw at 4 °C. **Do not thaw at 37 °C**.

2.1.2. In a biosafety cabinet, prepare **Differentiation Complete Medium A/B/C/D/E (1×)** according to Table 4.

Table 4: Preparation of hPSC–mDAP Differentiation Kit Complete Media

Type	Component	Final Concentration
Differentiation Complete Medium A/B/C/D/E (1×)	mDAP Differentiation Supplement A/B/C/D/E（100×）	1×
	mDAP Differentiation Basal Medium F	

2.1.3. Differentiation media are recommended to be **freshly prepared**. Store at 4 °C and use within 2 weeks.

Tips: Supplement A/B/C/D/E may be aliquoted and stored frozen according to experimental needs. The total number of freeze–thaw cycles should not exceed 2.

2.2. Differentiation of mDAPs

2.2.1. Culture and Preparation of hPSCs: Refer to the hPSC Culture Medium Instructions for Use for detailed procedures.

(https://www.shownin.com/download/8.html?page=1,hPSC Culture Medium Instructions)

2.2.2. **Day 0:** Using a 6-well plate as an example, when hPSCs reach approximately 85% confluence, aspirate the culture medium and rinse once with 2 mL/well of DPBS (without calcium and magnesium). Add 2 mL/well of **Solase Cell Dissociation Solution** and incubate at 37 °C in a humidified incubator with 5% CO_2 for 5–8 min. Gently rock the plate to facilitate complete detachment of cells from the matrix.

2.2.3. Transfer the cell suspension into a 1.5 mL microcentrifuge tube and centrifuge using a mini centrifuge for 10–15 s. Carefully aspirate and discard the supernatant. Collect the cells for counting, and transfer 2 × 10^6 hPSCs into 8 mL of **pre-warmed Differentiation Complete Medium A**. Add 8 µL **Blebbistatin (10 mM)** at a 1:1000 dilution and mix gently. Seed the cell suspension at 2 mL/well into 4 Matrigel-coated wells of a 6-well plate.

Tips：Using a 6-well plate as an example, the recommended seeding density for hPSCs is 5 × 10^5 cells/well. The same procedure may be applied to other vessel formats, maintaining a seeding density of 5 × 10^4 cells/cm^2.

2.2.4. **Day 1:** Aspirate **Differentiation Complete Medium A** and replace with 2 mL/well of **Differentiation Complete Medium B**. Replace the medium daily and proceed until Day 3 (Day 1–3).

2.2.5. **Day 3:** Aspirate **Differentiation Complete Medium B** and replace with 2 mL/well of **Differentiation Complete Medium C**. Replace the medium daily and proceed until Day 5 (Day 3–5).

2.2.6. **Day 5:** Aspirate **Differentiation Complete Medium C** and replace with 2 mL/well of **Differentiation Complete Medium D.** Replace the medium daily and proceed until Day 7 (Day 5–7).

2.2.7. **Day 7:** Aspirate **Differentiation Complete Medium D** and replace with 2 mL/well of **Differentiation Complete Medium E**.Replace the medium daily and proceed until Day 11 (Day 7–11).

2.2.8. **Day 11:** Aspirate **Differentiation Complete Medium E** and rinse once with 2 mL/well of DPBS (without calcium and magnesium). Add 1 mL/well of **Solase Cell Dissociation Solution** and incubate at 37 °C in a humidified incubator with 5% CO_2 for 8–10 min.Once the cells detach from the matrix, add 1 mL/well of DPBS (without calcium and magnesium) to resuspend the cells and collect the suspension. Centrifuge at 178 × g for 5 min, discard the supernatant, and collect the resulting cells. The harvested cells are mDAPs. The obtained progenitor cells may be used directly for further maturation or subjected to cryopreservation.

2.2.9. For cryopreservation, resuspend the cells in 1 mL **mDAP Cryopreservation Medium G** and determine cell density. Adjust the cell concentration to 5 × 10^6 cells/mL, aliquot 1 mL/cryovial, and label appropriately. Place the cryovials into a controlled-rate freezing container and store at -80 °C. On the following day, transfer the vials to liquid nitrogen for long-term storage. For maturation of dopaminergic neural progenitor cells, refer to next section. **(Section III: Maturation of hPSC-Derived Dopaminergic Neurons)**.

III. Maturation of hPSC-Derived Dopaminergic Neurons

3.1. Coating of Plates for Dopaminergic Neuron Maturation (24-Well Plate as an Example)

3.1.1. Poly-L-ornithine / Laminin (PLO/Laminin) Coating

3.1.1.1. **Reagent Preparation：PLO/Laminin-coated plates** are used for the maturation of mDAPs.

Product	Brand	Cat.No.	Concentration
Poly-L-ornithine	Sigma	P3655	10 mg/mL
Laminin	Sigma	L2020	1 mg/mL

3.1.1.2. Prepare 12 mL of pre-chilled sterile water in a 15 mL centrifuge tube. Add 4.8 µL **Poly-L-ornithine (10 mg/mL)** and mix thoroughly. Immediately dispense the solution into a 24-well plate at 500 µL/well, and store at 4 °C;

3.1.1.3. On the following day, remove the PLO-coated 24-well plate from 4 °C and allow it to equilibrate to room temperature. Aspirate the Poly-L-ornithine solution and rinse once with 1× DPBS;

3.1.1.4. Prepare 12 mL of pre-chilled sterile 1× DPBS in a 15 mL centrifuge tube. Add 48 µL **Laminin** (1 mg/mL) and mix thoroughly. Immediately dispense the solution into the 24-well plate at 500 µL/well, and store at 4 °C.

3.1.2. ncLaminin511 Coating

3.1.2.1. Reagent Preparation： ncLaminin511-coated plates are used for the maturation of mDAPs.

Product	Brand	Cat.No.	Concentration
ncLaminin511	Shownin	RP01025	100 µg/ mL

3.1.2.2. Prepare 12 mL of pre-chilled sterile 1× DPBS in a 15 mL centrifuge tube. Add 240 µL **ncLaminin511** (100 µg/mL) and mix thoroughly. Immediately dispense the solution into a 24-well plate at 500 µL/well, and store at 4 °C.

3.2. Maintenance of Dopaminergic Neural Progenitor Cells (hPSC–mDAP)

3.2.1. Preparation of mDAP Maintenance Medium

3.2.1.1. Thaw **mDAP Maintenance Supplement (100×)** at 4 °C.

3.2.1.2. In a biosafety cabinet, prepare the **mDAP Maintenance Medium** according to the following formulation:
mDAP Maintenance Basal Medium: 99 mL
mDAP Maintenance Supplement (100×): 1 mL

3.2.1.3. The prepared medium is recommended to be **freshly prepared**. Store at 4 °C and use within 2 weeks. The Supplement may be aliquoted and stored frozen according to experimental needs. The total number of freeze-thaw cycles should not exceed 2.

Table 5： Product Information for mDAP Maintenance Medium

Product	Cat.No.	Amount	Storage
hPSC-mDAP Maintenance Medium:	**RP01017-H**	**100 mL**	
mDAP Maintance Basal Medium	RP01017-H-01	99 mL	2–8 °C
mDAP Maintance Supplement （100×）	RP01017-H-02	1 mL	-80 °C to -20 °C

3.2.2. Recovery of mDAPs

3.2.2.1. Pre-warm a water bath to 37 °C. Place Matrigel- or ncLaminin511- coated 6-well plates in a biosafety cabinet for approximately 30 min to allow equilibration to room temperature.

3.2.2.2. Prepare an appropriate volume of **mDAP Maintenance Medium** and add **Blebbistatin** at a 1:2000 dilution (final concentration 5 µM). Allow the medium to equilibrate to room temperature before use.

3.2.2.3. Remove one vial of cryopreserved **hPSC-derived dopaminergic neural progenitor cells** from liquid nitrogen and transfer it on dry ice to the cell processing area. Immediately place the vial into a 37 °C water bath and gently agitate by hand. Complete thawing within 1 min, and remove the vial when ice crystals in the suspension are almost completely dissolved.

3.2.2.4. Disinfect the exterior of the cryovial with 75% ethanol and transfer it into a biosafety cabinet. Transfer the cell suspension into a pre-prepared 15 mL centrifuge tube. Using a pipette, slowly add 10 mL DMEM/F12 dropwise while gently swirling to mix. Centrifuge at 178 × g for 5 min.

3.2.2.5. Aspirate and discard the supernatant. Gently resuspend the cell pellet in 1 mL pre-warmed **mDAP Maintenance Complete Medium (+ Blebbistatin)**, minimizing pipetting to avoid mechanical stress. Take an aliquot of the suspension for cell counting.

3.2.2.6. Remove the coating solution from the 6-well plate. Seed the cells at a density of **1 × 10^5** cells/cm^2 using **0.2 mL/cm^2** medium in the corresponding wells. Gently rock the plate in a horizontal cross pattern 3 times to ensure even distribution and label accordingly. Place the plate into a 37 °C incubator with 5% CO_2 and saturated humidity, then gently rock again in a horizontal cross pattern 3 times before incubation.

3.2.2.7. Replace the medium with fresh **mDAP Maintenance Complete Medium** (3 mL/well) after 18–24 h. Subsequently, replace the medium every 2 days.On Day 4–5 **post-recovery, c**ollect the cells for counting using **Solase Cell Dissociation Solution (5–8 min)** for subsequent maturation.

Tips： This protocol describes mDAP recovery using a 6-well plate as an example and is applicable to other vessel formats. If continuous maturation is selected at Day 11 of hPSC-to-mDAP differentiation (Section 2.2.8), mDAP passaging may be performed using the same seeding density as described in Section 3.2.2.6.

3.3. Maturation of mDANs

Table 6： Product Information for Dopaminergic Neuron Maturation Differentiation Medium

Product	Cat.No.	Amount	Storage
Dopaminergic Neuron Maturation Differentiation Medium*contains:	**RP01017-I**	**50 mL**	
mDAN Maturation Basal Medium	RP01017-I-01	50 mL	2–8 °C
mDAN Maturation Supplement （100×）	RP01017-I-02	0.5 mL	-80 °C to -20 °C

3.3.1. Preparation of Dopaminergic Neuron Maturation Differentiation Medium

3.3.1.1. Thaw **mDAN Maturation Supplement (100×)** at 4 °C.

3.3.1.2. In a biosafety cabinet, prepare the **Dopaminergic Neuron Maturation Differentiation Medium** according to the following formulation:

mDAN Maturation Basal Medium: 50 mL

mDAN Maturation Supplement (100×): 0.5 mL

3.3.1.3. The maturation differentiation medium is recommended to be **freshly prepared**. Store at 4 °C and use within 2 weeks.

3.3.2. Dopaminergic Neuron Maturation Procedure

3.3.2.1. Place PLO/Laminin- or ncLaminin511- coated 24-well plates in a biosafety cabinet for approximately 30 min to allow equilibration to room temperature.

3.3.2.2. Prepare an appropriate volume of **mDAP Maintenance Medium** and add **Blebbistatin** at a 1:2000 dilution (final concentration 5 μM). Allow the medium to equilibrate to room temperature before use.

3.3.2.3. Collect dopaminergic neural progenitor cells obtained on Day 4–5 as described in **Section 3.2.2.7**. Resuspend the cells in **mDAP Maintenance Medium** and seed at a density of 5 × 10^5 cells/cm^2 onto Laminin- coated 24-well plates. Label accordingly and gently rock the plate in a horizontal cross pattern 3 times to ensure even distribution. Incubate at 37 °C in a humidified incubator with 5% CO_2.

3.3.2.4. Replace the medium with fresh **Dopaminergic Neuron Maturation Differentiation Medium** (0.5 mL/well) after 18–24 h. For the first 7 days, replace the medium daily. Thereafter, replace the medium every 2 days using 0.5 mL/well.

3.3.3. Characterization of Mature Dopaminergic Neurons

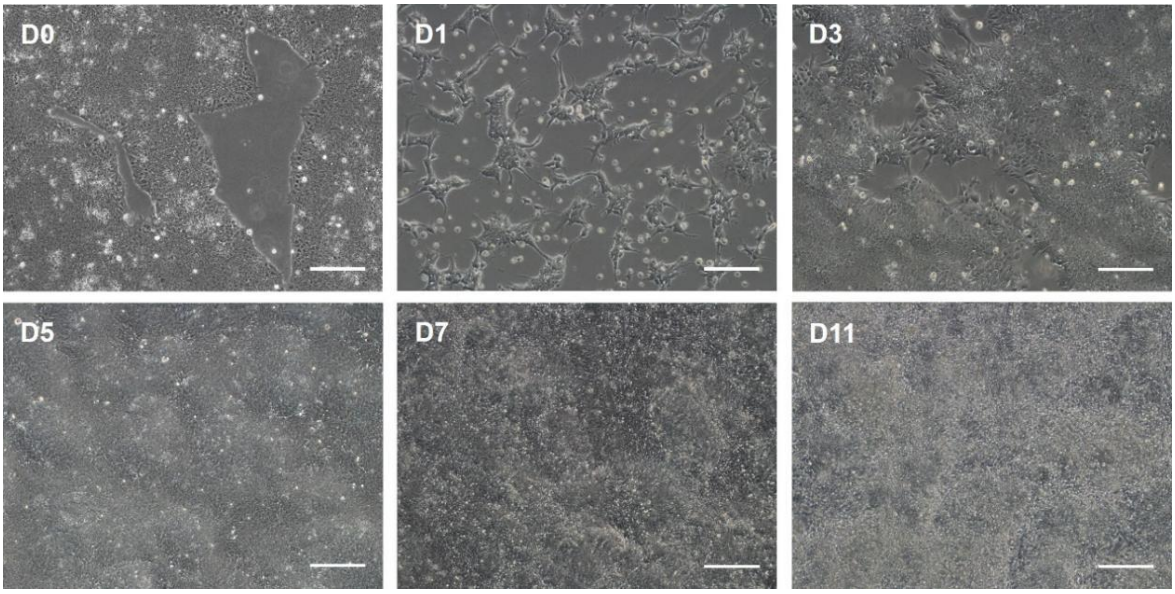
3.3.3.1. Place PLO/Laminin- or ncLaminin511- coated 24-well plates in a biosafety cabinet for approximately 30 min to allow equilibration to room temperature.

3.3.3.2. After approximately 20–30 days of maturation in **Dopaminergic Neuron Maturation Differentiation Medium**, dopaminergic neurons may be subjected to characterization of maturation markers.
For downstream assays, dissociate the cells into single-cell suspensions using **TrypLE** (37 °C, 10–15 min; do not exceed 15 min).

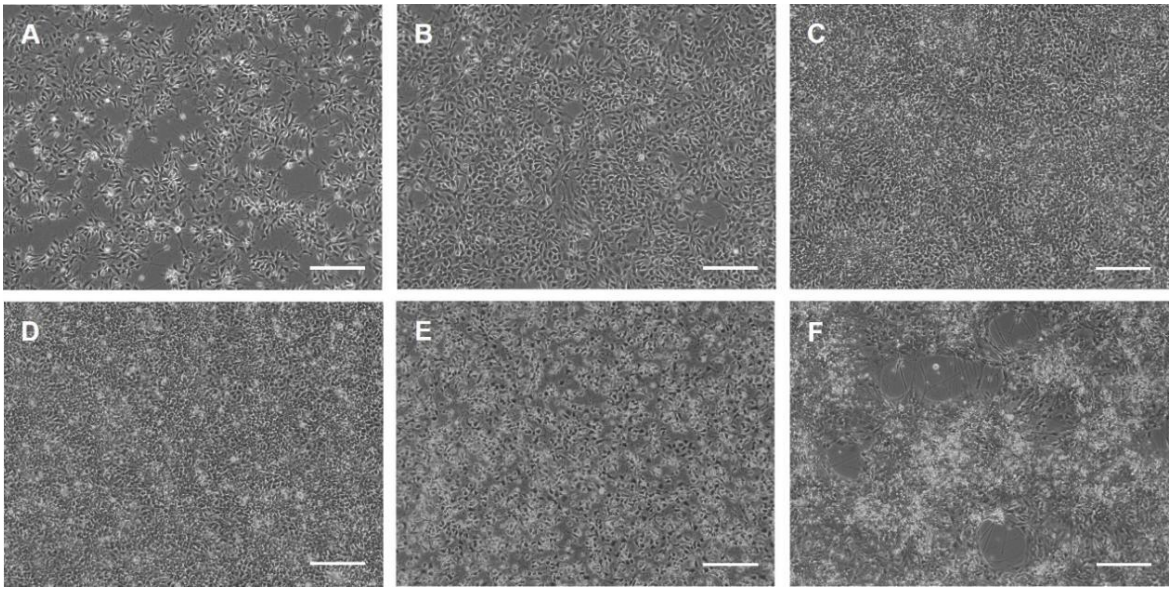
3.3.3.3. qPCR Analysis: Extract RNA from at least 2 × 10^6 cells, followed by qPCR analysis for dopaminergic neuron–specific markers.

3.3.3.4. Immunofluorescence Staining Analysis: Seed dopaminergic neurons at a density of 1–2 × 10^5 cells/cm² onto Laminin- coated plates and maintain for at least 3–5 days to allow morphological recovery prior to immunofluorescence staining.

3.3.3.5. Electrophysiological Analysis: Dopaminergic neurons matured for 40–50 days may be used for electrophysiological studies. Prior to electrophysiological recordings, maintain the neurons in **Dopaminergic Neuron Electrophysiology Medium (RP01017-J)** for 3 days.



Morphological progression during differentiation of hPSC-derived dopaminergic neural progenitor cells. Scale bar: 200 µm.
Representative phase-contrast images illustrating the differentiation process of hPSC-derived dopaminergic neural progenitor cells (mDAPs) at Day 0, 1, 3, 5, 7, and 11.



Morphology of hPSC-derived dopaminergic neural progenitor cells during recovery, maintenance, and maturation.
Images A–C show the morphology of mDAPs at Day 1, Day 2, and Day 4 following recovery, respectively.
Scale Bar: 200 μm
Images D–F show the morphological changes during differentiation of mDAPs into mature dopaminergic neurons (mDANs) at Day 1, Day 9, and Day 30, respectively.