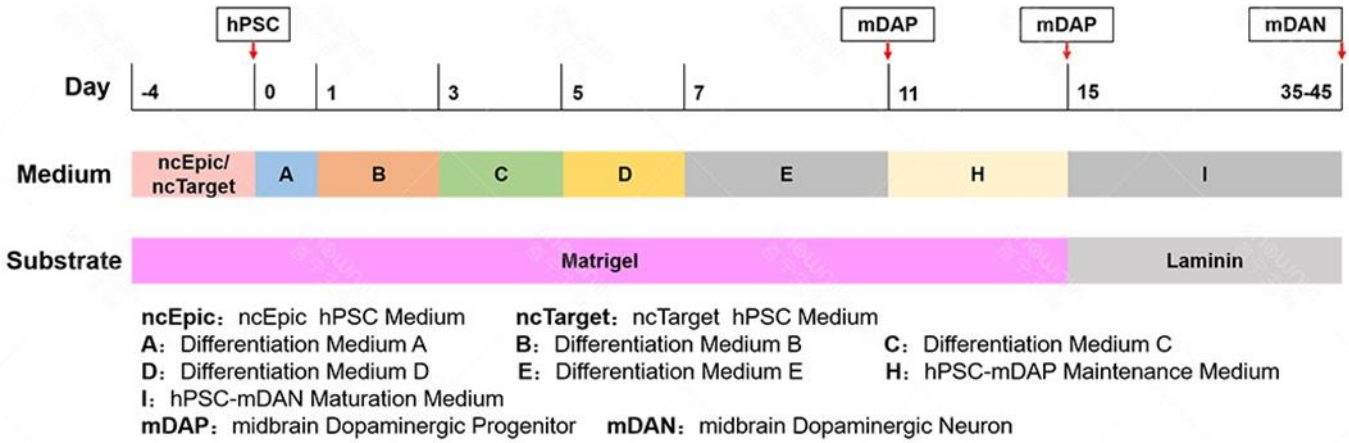
hPSC-mDAP Differentiation Kit

Product Manual V3

I. Product Introduction

1.1 Product Description

The hPSC-mDAP Kit is suitable for the differentiation of human pluripotent stem cells (hPSC) into mature dopaminergic neurons (mDAN, midbrain Dopaminergic Neuron). The kit contains Precursor Cell Differentiation Kit, Precursor Cell Maintenance Medium and Neuron Mature Differentiation Medium, which can be flexibly selected according to specific needs. The hPSC-dopaminergicPrecursor Cell Differentiation Kit can be used to obtain high-purity mDAP (>90% Lmx1a+/ Foxa2+/ En1+)from hPSC; the dopaminergic Neuron Mature Differentiation Medium can be used to differentiate mDAP into mature mDAN (TH+/Nurr1+) . The human dopaminergic neural precursor cells and human dopaminergic neurons obtained by differentiation can be applied to scientific research on neurodegenerative diseases, drug screening, and cell transplantation in Parkinson's disease models.



1.2 Product Information

Table 1: hPSC-mDAP Differentiation Kit Product Description

Product Information	Cat. No.	Amount	Storage
hPSC-mDAP Differentiation Kit*	RP01017	1 Kit	Basal Medium 2~8 Supplements -20~-80
hPSC-mDAP Maintenance Medium	RP01017-H	100 mL	
hPSC-mDAN Maturation Medium	RP01017-I	50 mL	
hPSC-mDAP	RC01008	1 x 10^6	liquid nitrogen

*Each kit can yield over 1×10^7 dopaminergic neural progenitor cells (mDAP, DAY11).

*The complete differentiation medium is prepared by mixing the basal medium and additives, and can be stored at 2 - 8°C and used up within 2 weeks

1.3 Recommended Reagents and Materials

Table 2: Recommended Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat. No. (e.g.)
NcEpic hPSC Medium	Shownin	RP01001
NcTarget hPSC Medium	Shownin	RP01020
Vitronectin	Shownin	RP01002
Research-grade hiPSC Line	Shownin	RC01001
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin	Shownin	RP01008
hPSC Cryopreservation Medium	Shownin	RP01003
Solase Cell Digestion Solution	Shownin	RP01021
ncLaminin511	Shownin	RP01025
TrypLE	Gibco	12604013
CORNING® MATRIGEL® Matrix	Corning	354277
DMEM/F12 Medium	ThermoSci.	11330
DPBS, no calcium, no magnesium	ThermoSci.	14190144
6-Well Plate	ThermoSci.	140685
1 mL/ 5 mL/ 10 mL/ 25 mL Pipettes	ThermoSci.	N/A
15 mL/ 50 mL Centrifuge Tubes	ThermoSci.	N/A
1.5/ 2 mL Cryovials	ThermoSci.	N/A
1.5 mL EP Tubes	Axygene	N/A
10 µL / 200 µL / 1000 µL Tips	Rainin.	N/A
Freezing Container	ThermoSci.	5100-0001

II. Differentiation of hPSC-Dopaminergic Neural Progenitor Cells

2.1 Preparation of Reagents

Table 3: Product Information of hPSC-mDAP Cell Differentiation Kit

Product Information	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	-20°C or -80°C
mDAP Differentiation Supplement C (100×)	RP01017-C	200 µL	-20°C or -80°C
mDAP Differentiation Supplement D (100×)	RP01017-D	200 µL	-20°C or -80°C
mDAP Differentiation Supplement E (100×)	RP01017-E	600 µL	-20°C or -80°C
mDAP Differentiation Basal Medium F	RP01017-F	120 mL	2□~8□
mDAP Cryopreservation Medium G	RP01017-G	50 mL	2□~8□

*More than 1×10^7 midbrain dopaminergic progenitor cells (mDAP, Day 11) can be obaiEach kit can be used for the differentiation of 8 wells in a 12-well plate or 4 wells in a 6-well plate.

*The complete differentiation medium is prepared by mixing the basal medium and additives, and can be stored at 2 - 8°C and used up within 2 weeks.

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

2.1.2. In a biosafety cabinet, prepare the differentiation complete media A/B/C/D/E (1×) according to Table 4.

Table 4: Instructions for Preparing the Complete Differentiation Medium

Type	Components	Final Concentration
Complete Differentiation 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. The differentiation medium should be freshly prepared and stored at 4°C. It is recommended to use it within two weeks.

TIPS: Supplements A/B/C/D/E can be aliquoted and frozen according to actual usage. The total number of freeze-thaw cycles should not exceed two.

2.2 Differentiation of Human Dopaminergic Neural Progenitor Cells

2.2.1 Culture and Preparation of hPSCs: For detailed instructions, refer to

the hPSC culture medium manual(http://www.nuwacell.com/list.php?pid=4&ty=20) and video tutorial (http://www.nuwacell.com/list.php?pid=4&ty=21).

2.2.2 On DAY 0, taking the operation of a 6-well plate as an example, when the confluence of hPSC cells reaches 85%, aspirate and discard the medium. Add 2 mL/well of DPBS (calcium- and magnesium-free) for 1 wash. Then add 2 mL/well of Solase cell digestive solution, and incubate it in an incubator at 37°C with a 5%CO2 concentration and saturated humidity for 5~8 min. Gently shake the well plate to make the cells completely detach from the substrate.

2.2.3 Transfer the cell suspension into a 1.5mL centrifuge tube and centrifuge using a benchtop centrifuge for 10~15 seconds. Carefully remove the supernatant, count the cells, and resuspend 2 × 10^6 hPSCs in 8mL of pre-warmed Complete Differentiation Medium A. Add 8μL of 10mM Blebbistatin at a 1:1000 dilution ratio. Dispense 2mL of the cell suspension into each of four wells of a Matrigel-coated 6-well plate.

TIPS: Taking the operation of a 6-well plate as an example, the seeding density of hPSC is 5×10^5/well. The operation procedure is also applicable to other culture vessels, and the seeding density of hPSC is 5×10^4/ cm^2.

2.2.4 On DAY 1, aspirate and remove the complete differentiation medium A, and then add 2 mL/well of complete differentiation medium B. Replace the medium daily and culture until DAY 3 (DAY 1~3).

2.2.5 On DAY 3, aspirate and remove the complete differentiation medium B, and then add 2 mL/well of complete differentiation medium C. Replace the medium daily and culture until DAY 5 (DAY 3~5).

2.2.6 On DAY 5, aspirate and remove the complete differentiation medium C, and then add 2 mL/well of complete differentiation medium D. Replace the medium daily and culture until DAY 7 (DAY 5~7).

2.2.7 On DAY 7, aspirate and remove the complete differentiation medium D, and then add 2 mL/well of complete differentiation medium E. Replace the medium daily and culture until DAY 11 (DAY 7~11).

2.2.8 On DAY 11, aspirate and remove the complete differentiation medium E. Add 2 mL/well of DPBS (calcium- and magnesium-free) for 1 wash. Then add 1 mL/well of Solase cell digestive solution, and incubate it in an incubator at 37°C with a 5% CO2 concentration and saturated humidity for 8~10 min. When the cells detach from the bottom of the culture dish, add 1 mL/well of DPBS (calcium- and magnesium-free) to resuspend and collect the cells. Centrifuge at 178×g for 5 minutes, discard the supernatant, and the obtained cells are dopaminergic neural progenitor cells. The obtained progenitor cells can be further matured and differentiated or cryopreserved.

2.2.9 If cryopreservation is required, add 1 mL of mDAP Cryopreservation Medium G to resuspend the cells and count them. Adjust the cell density to 5×105 cells/mL, and aliquot 1 mL/tube into cryotubes. Mark them properly. Transfer the cryotubes to a gradient cryopreservation box and place them in an -80°C refrigerator. Transfer them to a liquid nitrogen tank for long-term storage on the next day. For the maturation of dopaminergic neural progenitor cells, please refer to the next section (III. Maturation of hPSC-Dopaminergic Neurons).

III. Maturation of hPSC-mDAN

3.1 Coating of Culture Plates for the Maturation of hPSC-mDAN (Taking 24-well plate as an example)

3.1.1 **Coating with Poly-L-ornithine/Laminin (PO/Laminin)**

3.1.1.1 Preparation of Reagents: PO/Laminin-coated culture plates are used for the maturation culture of mDAP.

Product Information	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-cooled sterile water in a 15 mL centrifuge tube. Add 4.8 µl of Poly-L-ornithine (10 mg/mL), mix thoroughly, and quickly add 500 µl to each well of a 24-well plate. Store at 4°C.

3.1.1.3 On the next day, take out the 24-well plate coated with Poly-L-ornithine from 4°C, let it return to room temperature, discard the Poly-L-ornithine, and wash it once with 1x DPBS.

3.1.1.4 Prepare 12 mL of pre-cooled sterile 1x DPBS in a 15 mL centrifuge tube. Add 48µl of Laminin (1mg/mL), mix thoroughly, and quickly add 500 µl to each well of the 24-well plate. Store at 4°C.

3.1.2. **Coating with ncLaminin511**

3.1.2.1. Preparation of Reagents: ncLaminin511-coated culture plates are used for the maturation culture of mDAP.

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

3.1.2.2. Prepare 12 mL of pre-cooled sterile 1x DPBS in a 15 mL centrifuge tube. Add 240 µl ncLaminin511 (100 µg/mL), mix well, and quickly add 500 µl to each well of the 24-well plate. Store at 4°C.

3.2 Maintenance Culture of hPSC-mDAP

3.2.1 **Preparation of Dopaminergic Neuron Progenitor Cell Maintenance Medium**

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

3.2.1.2 In a biosafety cabinet, prepare the dopaminergic neural progenitor cell maintenance medium according to the following list.

mDAP Maintenance Basal Medium: 99 mL

mDAP Maintenance Supplement (100×): 1 mL

3.2.1.3 It is recommended to prepare the differentiation medium freshly and store it at 4°C. Use it within 2 weeks. The supplement can be aliquoted and stored frozen according to actual usage. The total number of freeze-thaw cycles should not exceed 2 times.

Table 5: Product Details of mDAP Maintenance Medium

Product Information	Cat. No.	Amount	Storage
mDAP Maintenance Medium	RP01017-H	100mL	10 mg/ mL
mDAP Maintance Basal Medium		99mL	1 mg/ mL

mDAP Maintance Supplement （100×）		1mL	
hPSC-mDAP	RC01008	1×10^6	liquid nitrogen

3.2.2 **Resuscitation of DAP Cells**

3.2.2.1 Preheat the water bath to 37°C. Place a Matrigel- or ncLaminin511-coated 6-well plate in a biosafety cabinet for about 30 minutes to return to room temperature.

3.2.2.2 Take an appropriate amount of dopaminergic neural progenitor cell maintenance medium, add Blebbistatin at a ratio of 1:2000 (final concentration 5 μM), and let it return to room temperature.

3.2.2.3 Wipe the surface of the cryopreservation tube with 75% alcohol - soaked dust-free paper and transfer it to a biosafety cabinet. Transfer the cell suspension into a pre-prepared 15mL centrifuge tube. Pipette 10 mL of DMEM/F12 and add it dropwise to the cryopreserved cell suspension while gently shaking to mix the cells. Centrifuge at 178×g for 5 minutes.

3.2.2.4. Discard the supernatant, add 1 mL of pre-warmed complete dopaminergic neural progenitor cell maintenance medium (+ Blebbistatin) to mix the cells. Avoid pipetting as much as possible. Take an appropriate amount of cells for counting.

3.2.2.5. Aspirate the coating solution in the 6 - well plate. Seed the cells into the corresponding wells at a density of 1×10^5 cells/cm² with a medium volume of 0.2 mL/cm². Shake the plate horizontally in a cross-pattern 3 times and label it properly. Place the plate in an incubator at 37°C with a 5% CO_2 concentration and saturated humidity, and shake it horizontally in a cross-pattern 3 times again before culturing.

3.2.2.6. Replace the complete maintenance medium for dopaminergic neural progenitor cells with fresh medium 18~24 hours later, at a volume of 3 mL per well. Subsequently, change the medium every 2 days. Collect and count the cells on days 4~5 (using Solase cell digestive solution for 5~8 min) for subsequent maturation and differentiation.

TIPS: This instruction takes the resuscitation of mDAP in a 6-well plate as an example, and it is also applicable to other culture vessels. When hPSC-dopaminergic neural progenitor cells reach day 11 of differentiation, if continuous maturation and differentiation is chosen (2.2.8), sub-culture of mDAP can be carried out at the same seeding density as in 3.2.2.6

3.3 Maturation of mDAN

Table 6: Product Details of mDAN Maturation Differentiation Medium

Product Information	Cat.No.	Norm	Storage
mDAN Maturation Differentiation Medium :	**RP01017-I**	**50 mL**	
mDAN Maturation Basal Medium		50 mL	2℃8℃
mDAN Maturation Supplement (100×)		0.5 mL	-20°C ~ -80°C

3.3.1. **Preparation of mDAN Maturation Medium**

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

3.3.1.2 In a biosafety cabinet, prepare the mDAN Maturation Medium as follows:

mDAN Maturation Basal Medium: 49.5 mL

mDAN Maturation Supplement (100×): 0.5 mL

3.3.1.3 It is recommended to prepare the maturation medium fresh and use it within two weeks. The Supplement can be aliquoted and stored at -20°C to -80°C. The total number of freeze-thaw cycles should not exceed 2.

3.3.2. **Maturation Culture of mDAN**

3.3.2.1 For 24-well plates coated with Poly-L-ornithine/Laminin (PO/Laminin) or ncLaminin511, place them in the biosafety cabinet for about 30 minutes to equilibrate to room temperature.

3.3.2.2 Take an appropriate amount of dopaminergic neural precursor cell maintenance medium and add Blebbistatin (final concentration 5 μM) at a ratio of 1:2000, allowing the mixture to equilibrate to room temperature.

3.3.2.3 Seed dopamine neural progenitor cells collected on Day 4~5 of Step 3.2.2.7 into 24-well plates pre-coated with Laminin at a density of 5×10^5 cells/cm². Resuspend the cells in Dopaminergic Neural Progenitor Cell Maintenance Medium. Label the wells, mix by cross-shaking three times, and incubate at 37 °C with 5% CO_2 under humidified conditions.

3.3.2.4 After 18–24 hours, replace the medium with fresh Dopaminergic Neuron Maturation Medium at 0.5 mL per well. Perform daily medium changes during the first 7 days, then change medium every 2 days at the same volume per well.

3.3.3 **Characterization of Mature Dopaminergic Neurons**

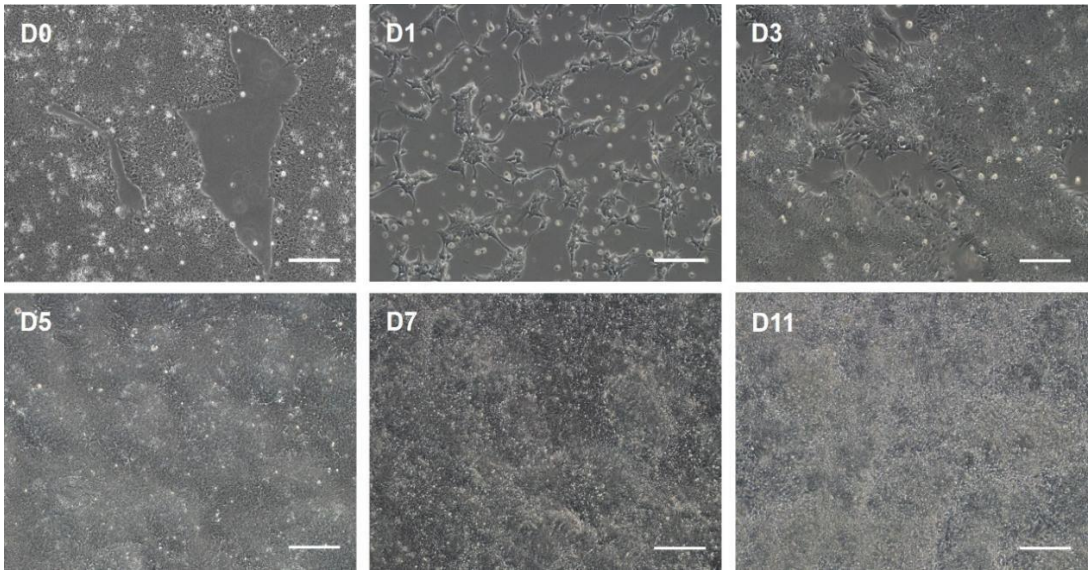
3.3.3.1 Place the PO/Laminin- or ncLaminin511-coated 24-well plate in a biosafety cabinet for 30 minutes to equilibrate to room temperature.

3.3.3.2 After 20-30 days of culture, mature dopaminergic neurons can be assessed for maturity markers. Use TrypLE digestion (37°C, 10~15 minutes, do not exceed 15 minutes) to process the cells into a single-cell suspension for subsequent analysis.

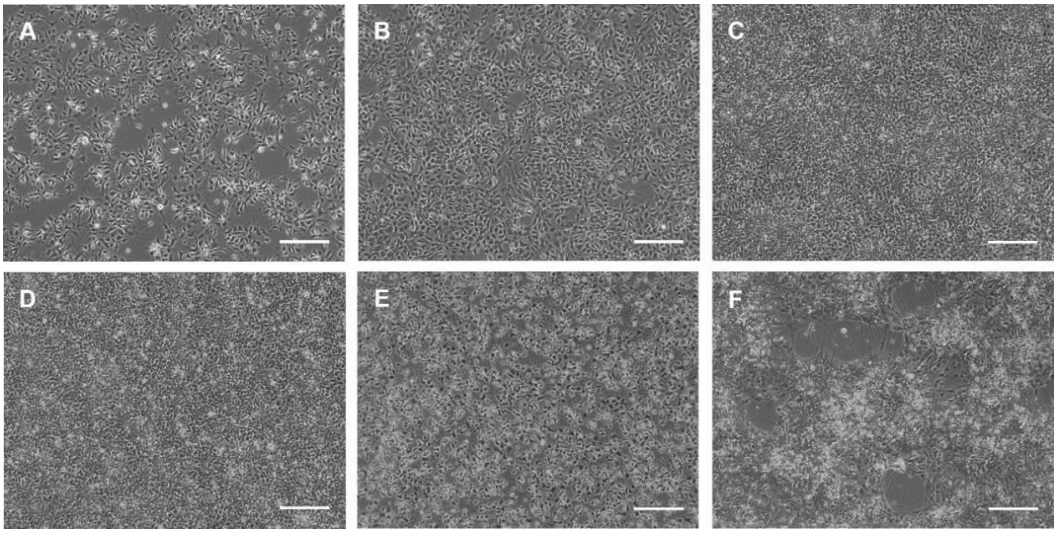
3.3.3.3 qPCR Identification: Extract RNA from at least 2×10^6 cells and perform relevant identification assays.

3.3.3.4 Immunofluorescence identification: Seed dopaminergic neurons at a density of $1–2 \times 10^5$ cells/cm² on Laminin-coated culture plates. Culture the cells for at least 3–5 days until morphological recovery is observed, then proceed with immunofluorescence staining.

3.3.3.5 Electrophysiological identification: Dopaminergic neurons cultured for 40–50 days can be used for electrophysiological studies. Prior to testing, incubate the cells in Dopaminergic Neuron Electrophysiology Medium (RP01017-J) for 3 days.



Morphology of hPSC-dopaminergic neural progenitor cells during the differentiation process using the hPSC-dopaminergic neural progenitor cell differentiation kit. Scale bar: 200 μm. The images are labeled as DAY 0, 1, 3, 5, 7, and 11.



Morphology of hPSC-dopaminergic neural progenitor cells during revival, maintenance culture, and maturation. Scale bar: 200 μm.

Figure 2A, B, C: Morphology of hPSC-dopaminergic neural progenitor cells (mDAP) on DAY 1, 2, and 4, respectively.

Figure 2D, E, F: Morphology of mDAP cells differentiating into mature dopaminergic neurons (mDAN) on DAY 1, 9, and 30, respectively.