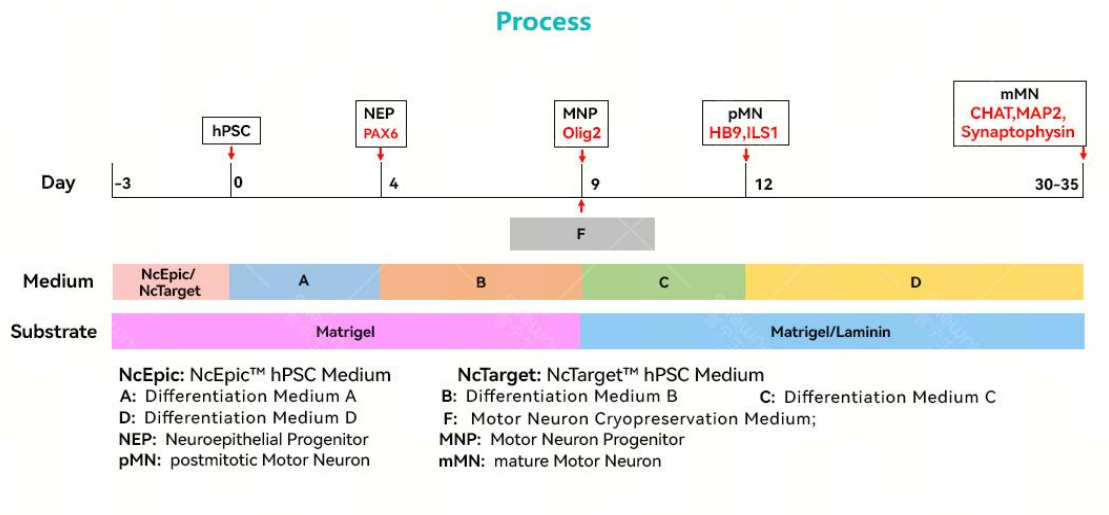
hPSC-Motor Neuron Differentiation Kit

Product Manual

Catalog#RP01018 1 Kit

Product Introduction

The hPSC-Motor Neuron Differentiation Kit is designed to efficiently direct the differentiation of human pluripotent stem cells (hPSCs) into motor neurons. The differentiated motor neurons express specific markers such as CHAT, MAP2, and Synaptophysin, and exhibit electrophysiological activity characteristic of motor neurons. This kit is suitable for in vitro studies and the establishment of disease-related experimental model.



Product Information

Table 1. hPSC-Motor Neuron Differentiation Product System

Product	Cat.No.	Amount	Storage
hPSC-Motor Neuron Differentiation Kit*	RP01018	1 Kit	Basal Medium 2–8 °C Supplements -80 °C or -20 °C
Motor Neuron Maturation Medium	RP01018-G	1 Kit	

***Each kit can differentiate and yield 1 × 10^7 motor neuron progenitor cells (MNP) by Day 9.**

***Mix the basal medium and additives evenly to prepare the differentiation medium, which can be stored at 2–8 °C and should be used up within two weeks.**

Reagents and Materials

Table 2. Recommended Reagents & Materials & Equipment

Reagents & Materials	Brand (e.g.)	Cat.No. (e.g.)
NcEpic™ hPSC Medium	Shownin	SN-01-0010
NcTarget™ hPSC Medium	Shownin	RP01020
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin	Shownin	RP01008
Solase Cell Dissociation Solution	Shownin	RP01021
ncLaminin511	Shownin	RP01025
Corning® Matrigel® Matrix	Corning	354277
Poly-L-ornithine	Sigma	P3655
Laminin	Sigma	L2020
TrypLE	Gibco	12604013
DMEM/F12 Medium	Thermo Sci.	11330
DPBS, no calcium, no magnesium	Thermo Sci.	14190144
12-Well Plate	Thermo Sci.	150628
T25 Culture Flasks	Thermo Sci.	156367
1 mL/5 mL/10 mL/25 mL Pipettes	Thermo Sci.	N/A
15 mL/50 mL Centrifuge Tube	Thermo Sci.	N/A
1.5/2 mL Cryovials	Thermo Sci.	N/A
1.5 mL EP Tubes	Axygene	N/A
10 µL/200 µL/1000 µL Pipette Tips	Rainin.	N/A
Freezing Container	Thermo Sci.	5100-0001

hPSC-Motor Neuron Differentiation

4.1 Reagents Preparation

Table 3. hPSC-Motor Neuron Differentiation Kit Product Description

Product Information	Cat.No.	Amount	Storage
The hPSC-Motor Neuron Differentiation Kit* contains:	**RP01018**	**1 Kit**	
Motor Neuron Differentiation Supplement A (20x)	RP01018-A	1 mL	-80 °C or -20 °C
Motor Neuron differentiation Supplement B (25x)	RP01018-B	1 mL	
Motor Neuron differentiation Supplement C (50x)	RP01018-C	1 mL	
Motor Neuron differentiation Supplement D (50x)	RP01018-D	3 x 1 mL	
Motor Neuron differentiation Basal Medium E	RP01018-E	2 x 125 mL	2–8 °C
Motor Neuron Cryopreservation Medium F	RP01018-F	20 mL	

***Each kit can be used to differentiate and obtain 1 × 10^7 motor neuron progenitor cells (Day 9, MNP).**

***Each kit can be applied to the differentiation of MNP (from Day 0 to Day 9) in 4 wells of a 12-well plate or 2 wells of a 6-well plate, as well as the maturation of MNP (from Day 9 to Day 30) in 10 wells of a 12-well plate or 20 wells of a 24-well plate.**

4.1.1 Thaw Motor Neuron Differentiation Supplement A/B/C/D at 4 °C**, do not thaw at 37 °C.**

4.1.2 In a biosafety cabinet, refer to the ratio provided in the Table 3, use a sterile pipettes and pipette Tips to mix to form **Complete Differentiation Medium A/B/C/D (1×)**.

4.1.3 It is recommended that the differentiation medium be prepared and **used immediately.** Store it at 4 °C and use it within two weeks.

Tips: Motor Neuron Differentiation Supplement A/B/C/D can be aliquoted according to the actual usage amount and then stored frozen. The total number of freeze-thaw cycles should not exceed 2 times.

Table 4. Instructions for Complete Medium Configuration

Types	Components	Final Volume
Complete Differentiation Medium A/B/C/D (1x)	Motor Neuron Differentiation Supplement A (20x)/B (25x)/C (50x)/D (50x)	1×
	Motor Neuron Differentiation Basal Medium E	

4.2 The hPSC-Motor Neuron Differentiation

4.2.1 Preparation of hPSC: For detailed instructions, please refer to the hPSC Culture Medium User Manual. (https://www.shownin.com/download/8.html?page=1, Operation Manual)

4.2.2 Day 0~4: Neuroepithelial Progenitor (NEP) Differentiation

4.2.2.1 Day 0, when the confluence of hPSCs in the culture dish reaches 80–90%, aspirate the hPSC **complete medium** (NcEpic or NcTarget), add 2 mL/well of DPBS (without calcium and magnesium), gently shake and then aspirate it.

4.2.2.2 Add 1 mL/well pre-warmed **Solase Cell Dissociationion Solution** to completely cover the bottom of the well, and then maintain it in an incubator with a temperature of 37 °C, a CO_2 concentration of 5% and saturated humidity for 5–7 minutes. Gently shake to detach cells.

4.2.2.3 Transfer the cell suspension into a 1.5 mL centrifuge tube and centrifuge it 10–15 seconds.

4.2.2.4 Aspirate the supernatant, add 1 mL of **Complete Differentiation Medium A** to resuspend the cells. Gently pipette 1–2 times to achieve single-cell suspension, then count the cell.

4.2.2.5 Seed 3–5 × 10^5 cells/well in a Matrigel-coated 6-well plate with 2 mL **Complete Differentiation Medium A** (containing 10 μM **Blebbistatin**).

4.2.2.6 Place plate at 37 °C, 5% CO_2, humidified incubator. Perform 3 gentle cross-shaped swirls (horizontal then vertical motions) to ensure even cell distribution and maintain plate under standard conditions.

4.2.2.7 After initial 22–24h, completely aspirate spent medium using sterile technique, add 2 mL/well fresh **Complete Medium A**. Place plate at 37 °C, 5% CO_2, humidified incubator and maintain it under standard conditions. Change the medium every Day.

Tips: When changing the medium on Day 1 and afterwards, do not add Blebbistatin to the Complete Differentiation Medium A.

4.2.3 Day 4~9: Motor Neuron Progenitor (MNP) Differentiation

4.2.3.1 Day 4, aspirate the medium, add 2 mL of **Complete Differentiation Medium B**. Place plate at 37 °C, 5% CO_2, saturated humidity atmosphere and maintain it under standard condition. Change the medium every Day (Day 4–9).

4.2.4 Day 9~12: Postmitotic Motor Neuron (pMN) Differentiation

4.2.4.1 Day 9, aspirate the medium, add 2 mL/well of DPBS (without calcium and magnesium), gently shake and then aspirate it.

4.2.4.2 Add 1 mL/well of pre-warmed **Solase Dissociationion Solution** to completely cover the bottom of the well. Incubate at 37 °C, 5% CO_2 and saturated humidity for 8–12 minutes. Gently shake the plate to make the cells completely detach.

Tips: If cells are not fully detached after 12 min, extend the incubation to 18–20 min (do not exceed 20 min).

4.2.4.3 Transfer the cell suspension into a 1.5 mL centrifuge tube and centrifuge it in a palm centrifuge for 10–15 seconds.

4.2.4.4 Aspirate the supernatant, add 1 mL of **Complete Differentiation Medium C** to resuspend the cells, gently pipette 1–2 times to achieve a single-cell suspension, then perform a cell count.

Tips: The harvested MNP cells can be cryopreserved at this stage. Resuspend in 1 mL Motor Neuron Cryopreservation Medium F at a density of 2×10^6 cells/tube.

4.2.4.5 Seed the cells at 2×10^5–6×10^5 cells/well into **Matrigel (2×)- or Laminin-** coated 12-well plates. Add 1 mL/well of **Complete Differentiation Medium C** (containing 10 μM of **Blebbistatin**).

4.2.4.6 Place the plate in an incubator with a temperature of 37 °C, a 5% CO_2 and gently swirl the plate 3 times in a cross pattern to ensure even distribution. Continue maintaining it under standard conditions.

4.2.4.7 After 22–24 hours, aspirate the medium replace with 1 mL/well **Differentiation Complete Medium C (without Blebbistatin)**. Maintain plate at 37 °C, 5% CO_2, saturated humidity atmosphere.

Change the medium every Day (Day 9–12).

Tips: Motor neurons adhere loosely, so it is recommended to coat the culture plate with 2× concentration of Matrigel. Additionally, from Day 10 onward, when changing the medium, handle the cells gently and make sure to remove Blebbistatin.

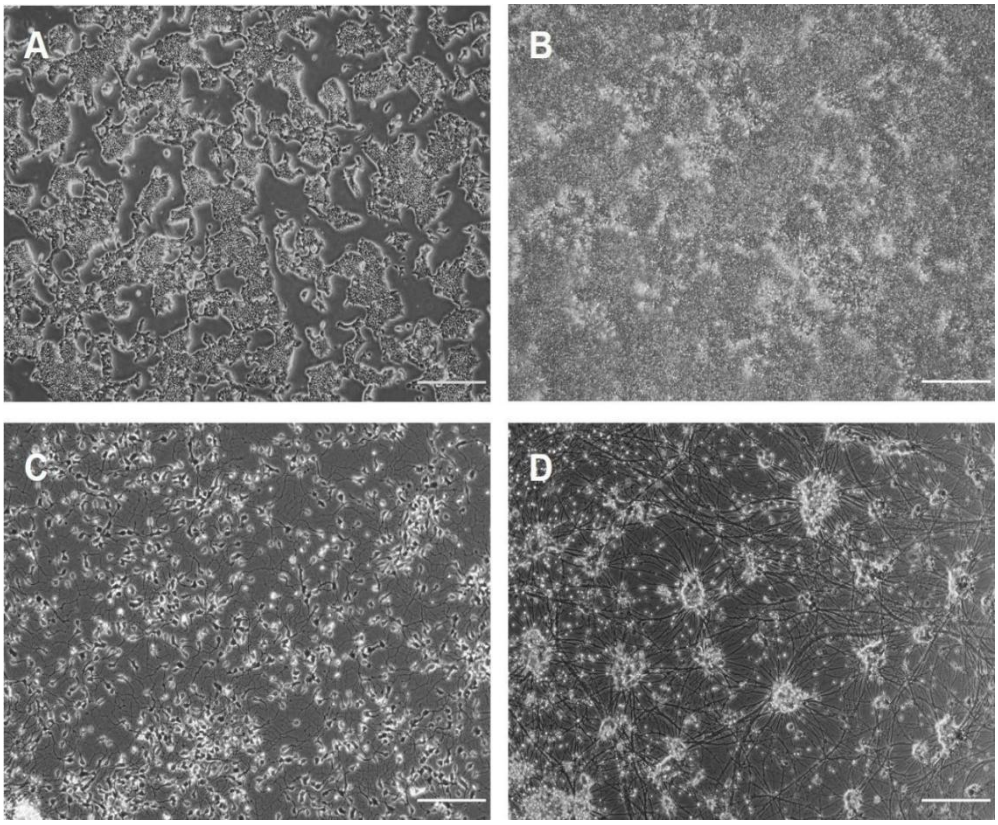
4.2.5 Day 12~35: Motor Neuron Maturation

4.2.5.1 Day 12, aspirate the cell culture medium, add 1 mL/well of **Complete Differentiation Medium D.** Place plate at 37 °C, 5% CO_2, saturated humidity atmosphere. Change the medium every Day.

Tips: Motor Neurons adhere loosely, so be especially gentle when changing the medium.

4.2.5.2 Mature Motor Neuron can be obtained at Day 30–35. Relevant identification assays can be performed.

4.2.5.3 Electrophysiological Identification: Culture cells for 3 Days in **Motor Neuron Electrophysiology Medium (RP01018-H)** prior to electrophysiological recording.



Morphology of hPSC-Motor Neuron Differentiation During the Differentiation Process. Scale bar: 200 µm. Figures A, B, C, and D show the morphology of cells on Days 4, 9, 12, and 30 of differentiation, respectively.

hPSC-motor neuron progenitor cell resuscitation and maturation culture

5.1 Reagent Preparation

Table 5. Motor Neuron Maturation Medium Product System:

Product Information	Cat.No.	Amount	Storage
Motor Neuron Maturation Medium* Contains	**RP01018-G**	**1 Kit**	
Motor Neuron Differentiation Supplement C (50×)	RP01018-C	1 mL	-80 °C or -20 °C
Motor Neuron Differentiation Supplement D (50×)	RP01018-D	3 x 1 mL	
Motor Neuron Differentiation Basal Medium E	RP01018-E	200 mL	2–8 °C

5.1.1. Thaw Motor Neuron Differentiation Supplement C and D at 4 °C, **do not thaw at 37 °C**.

5.1.2. In a biosafety cabinet, prepare **Complete Differentiation Medium C/D (1×)** with reference to Table 4.

5.1.3. The differentiation medium is recommended to **be prepared and used immediately.** Store it at 4 °C and use it within two weeks.

Tips: Motor Neruon Differentiation Supplement C/D can be aliquoted according to the actual usage amount and then stored frozen. The total number of freeze-thaw cycles should not exceed 2 times.

5.2 Application in hPSC-motor neuron progenitor cell resuscitation and maturation culture

5.2.1 Preheat the water bath to 37 °C. Place **Matrigel-** or **Laminin-** coated 6-well plates in a biosafety cabinet for approximately 30 minutes beforehand to return to room temperature.

5.2.2 Take an appropriate amount of **the complete differentiation medium C**, add **Blebbistatin** at a ratio of 1:1000 (final concentration of 10 μM), and allow it to return to room temperature.

5.2.3 Take out 1 tube of frozen **hPSC-motor neuron progenitor cells** from the liquid nitrogen tank, transfer them to the cell culture room on dry ice, immediately place them in a 37 °C water bath and gently shake them by hand. Thaw them within 1 minute. Take them out when it is observed with the naked eye that the ice crystals in the cell suspension are about to disappear completely.

5.2.4 Wipe the surface of the cryopreservation tube with 75% alcohol dust-free paper and transfer it into the biological safety cabinet. Transfer the cell suspension to a pre-prepared 15 mL centrifuge tube. Use a pipette to aspirate 10 mL of DMEM/F12 and add it drop by drop to the frozen cell suspension. During the process, gently shake to mix the cells evenly. Centrifuge at 178 × g for 5 minutes.

5.2.5 Aspirate the supernatant, add 1 mL of pre-warmed **Complete Differentiation Medium C** to mix the cells, try to avoid pipetting. Take an appropriate amount of cells for counting. Inoculate them into 12-well plates coated with **Matrigel** or **Laminin** at a density of **2×10^5–6×10^5 cells/well**. Place them in an incubator with a temperature of 37 °C, a CO_2 concentration of 5%, and saturated humidity. Shake them horizontally in a cross shape for 3 times and then maintain them under standard condition.

5.2.6 After maintaining for 22–24 hours, aspirate and discard the cell culture medium, add 1 mL/well of **Complete Differentiation Medium C,** place plate in an incubator with a temperature of 37 °C, a CO_2 concentration of 5%, and saturated humidity. Maintain uunder standard condition for 3 Days. At this time, the operation can be carried out with reference to **the steps in 4.2.4.**

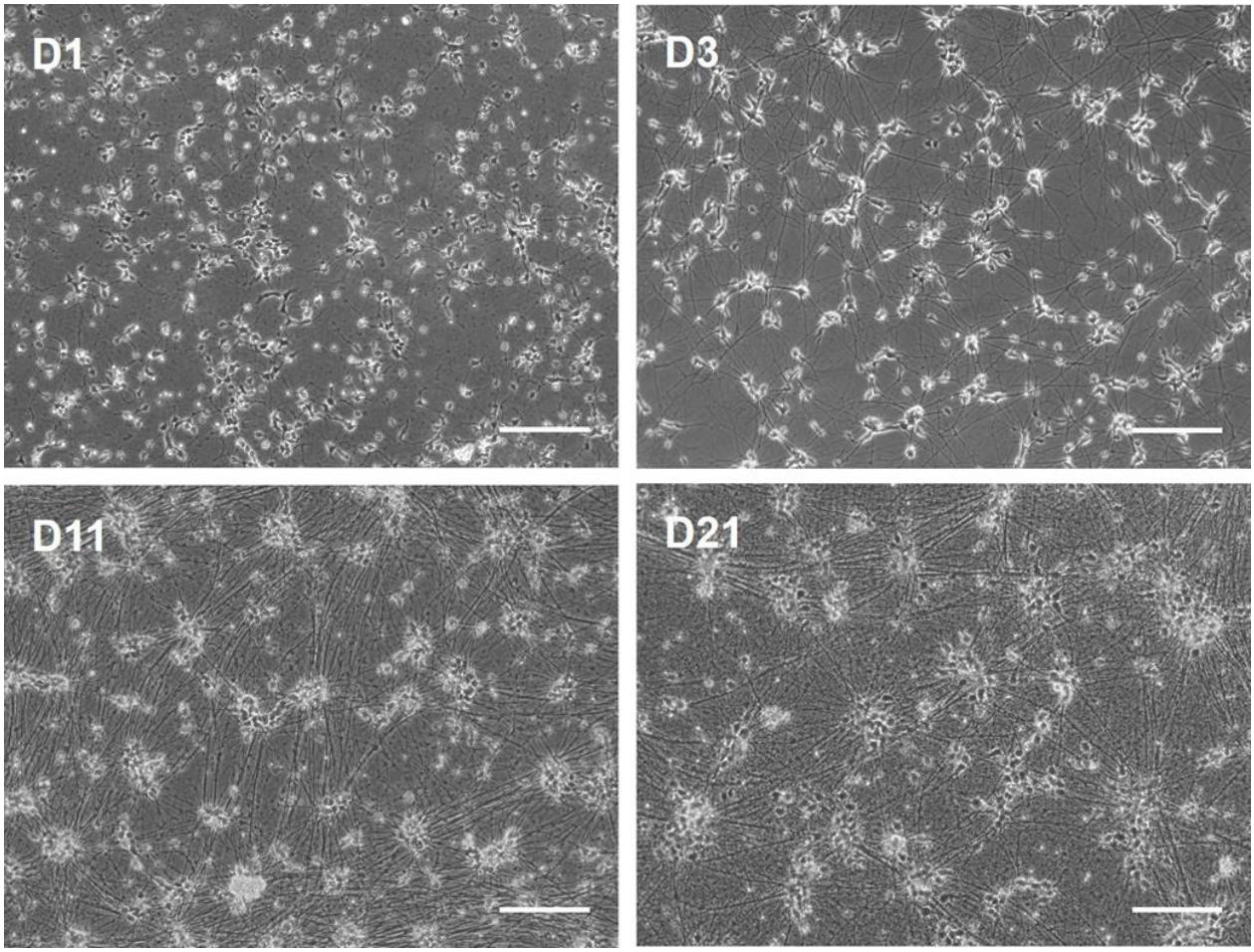
5.2.7 Day 3, aspirate the cell culture medium, add 1 mL/well of **Complete Differentiation Medium D**, place them in an incubator with a temperature of 37 °C, a CO_2 concentration of 5%, and saturated humidity. Change the medium every other Day.

Tips: Motor Neurons adhere loosely, so be especially gentle when changing the medium and remove Blebbistatin.

5.2.8 Mature Motor Neurons can be obtained in Day 18–23. Relevant identifications can be carried out. Treat them into single cells by Dissociationion with **TrypLE** (at 37 °C for 10–15 minutes, not exceeding 15 minutes) for subsequent detections.

5.2.9 qPCR Identification: Extract RNA from at least 2×10^6 cells and then conduct relevant identifications.

5.2.10 Electrophysiological Identification: When performing electrophysiological detection, it is necessary to culture the cells in **Motor Neuron Electrophysiology Medium (RP01018-H)** for 3 Days in advance.



Morphology of hPSC-Motor Neuron Progenitor Cells Post-Thawing (Day 1–Day 21)

Scale bar: 200 μm.