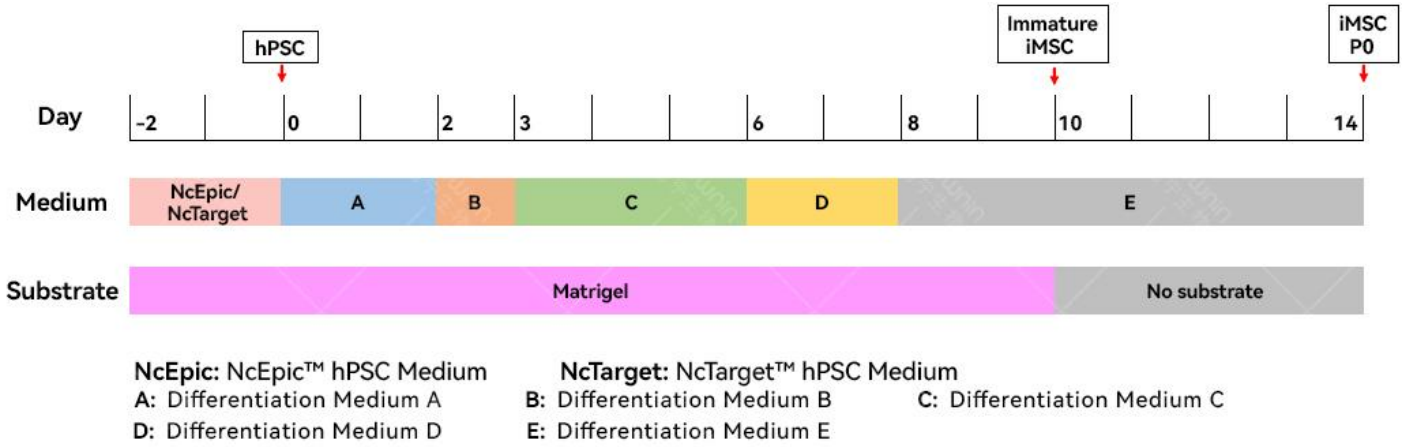
hPSC-MSC Differentiation Kit

Product Manual

I. Product Introduction

The hPSC-MSC Differentiation Kit is designed for efficient differentiation of human pluripotent stem cells (hPSCs) into high-purity mesenchymal stem cells (MSCs). The resulting MSCs exhibit stable proliferation, normal karyotype, characteristic surface marker expression (CD73+/CD90+/CD105+, CD14-/CD34-/CD45-/CD79a-/HLA-DR-), and trilineage differentiation potential (osteogenic, chondrogenic, and adipogenic). hPSC-MSCs are suitable for in vitro studies, drug screening, safety evaluation, and cell transplantation therapy in disease models.



II. Product Information

Table 1: Description of hPSC-MSC Differentiation Kit

Product Information	Cat. No.	Amount	Storage
hPSC-MSC Differentiation Kit* Contains:	**RP01013**	**1 Kit**	
MSC Differentiation Supplement A (10×)	RP01013-A	0.5 mL	-20°C or -80°C
MSC Differentiation Supplement B (10×)	RP01013-B	0.5 mL	-20°C or -80°C
MSC Differentiation Supplement C (10×)	RP01013-C	1 mL	-20°C or -80°C
MSC Differentiation Supplement D (10×)	RP01013-D	0.5 mL	-20°C or -80°C
MSC Differentiation Supplement E (30×)	RP01013-E	1 mL	-20°C or -80°C
MSC Differentiation Basal Medium F	RP01013-F	55 mL	2°C~8°C

***Each kit yields approximately 2×10^7 P0-generation MSCs.**

***Prepared differentiation complete medium can be stored at 2°C–8°C for up to 2 weeks.**

III. Reagent and Materials

Table 2: Recommended Reagents and Materials

Reagents & Materials	Brand (e.g.)	Gat.No. (e.g.)
Research-grade hiPSC Cell Line	Shownin	RC01001
NcEpic™ hPSC Medium	Shownin	RP01001
NcTarget™ hPSC Medium	Shownin	RP01020
hPSC Cryopreservation Medium	Shownin	RP01003
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin (10 mM)	Shownin	RP01008
ncMission hMSC Medium	Shownin	RP02010
MSC Cryopreservation Medium	Shownin	RP02004
Solase Cell Dissociation Solution	Shownin	RP01021
Corning® Matrigel® Matrix	Corning	354277
DMEM/F12 Medium	Thermo Sci.	11330
DPBS, no calcium, no magnesium	Thermo Sci.	14190144
6-Well Plates	Thermo Sci.	140685
1 mL/5 mL/10 mL/25 mL Pipettes	Thermo Sci.	N/A
15 mL/50 mL Centrifuge Tubes	Thermo Sci.	N/A
1.5/2 mL Cryovials	Thermo Sci.	N/A
10 µL/200 µL/1000 µL Pipette Tips	Rainin.	N/A
Freezing Container	Thermo Sci.	5100-0001

IV. Reagent Preparation

- **Preparation of hPSC-MSC Differentiation Complete Media**

1. Thaw MSC Differentiation Supplements A, B, C, D, and E at 4°C. **Do not thaw at 37°C.**

2. In a biosafety cabinet, prepare **Differentiation Complete Media A/B/C/D/E (1×)** according to **Table 3** using sterile pipettes and tips.
3. Prepared media should be used immediately or stored at 4°C for up to 2 weeks.
   TIPS: Aliquot MSC Differentiation Supplements for freezing if needed. Avoid more than 2 freeze-thaw cycles.

Table 3: Proportion of hPSC-MSC Differentiation Complete Medium

Medium Type	Components	Final Concentration
Complete Differentiation Medium-A	MSC Differentiation Basal Medium F	1×
	MSC Differentiation Supplement A (10×)	
Complete Differentiation Medium-B	MSC Differentiation Basal Medium F	1×
	MSC Differentiation Supplement B (10×)	
Complete Differentiation Medium-C	MSC Differentiation Basal Medium F	1×
	MSC Differentiation Supplement C (10×)	
Complete Differentiation Medium-D	MSC Differentiation Basal Medium F	1×
	MSC Differentiation Supplement D (10×)	
Complete Differentiation Medium-E	MSC Differentiation Basal Medium F	1×
	MSC Differentiation Supplement E (30×)	

V. hPSC-MSC Differentiation

5.1 **hPSC Culture and Preparation: Refer to the hPSC Medium Instruction Manual for details.**

Operation Manual: https://www.shownin.com/download/8.html?page=1

Video Tutorial: https://www.shownin.com/video.html

For a 6-well plate, seed hPSCs at 4×10^5 cells/well and culture continuously for 2 days.
TIPS: Use hPSCs after ≥5 passages for differentiation. For other vessels, seed at 4×10^4 cells/cm² with 200 µL medium/cm².

5.2 DAY -1, seed hPSCs into a new 6-well plate as described in 5.1 and culture continuously.

5.3 Day 0 (24–36 hours later), aspirate hPSC complete medium (NcEpic or NcTarget), wash twice with DMEM/F12, and then add 2 mL/well of Differentiation Complete Medium A.

5.4 DAY 2 (40 hours after Differentiation Complete Medium A change), aspirate **Complete Differentiation Medium A** and wash once with DMEM/F12. Then add 2 mL/well of **Complete Differentiation Medium B.**

5.5 Day 3 (24 hours later), aspirate **Complete Differentiation Medium B.** Then add 2 mL/well of **Complete Differentiation Medium C** (no need to wash with DMEM/F12)

TIPS: Steps 5.2–5.5 have strict timing requirements for media changes. The recommended schedule is as follows: Step 5.2: Passage at 11:00 AM; Step 5.3: 30 hours after passage (by 5:00 PM the next day), replace with Differentiation Complete Medium A; Step 5.4: On Day 4 at 9:00 AM, replace with Differentiation Complete Medium B.

5.6 DAY 4, Replace the medium with 2 mL/well of **Complete Differentiation Medium C** (no wash).

5.7 DAY 6, aspirate **Complete Differentiation Medium C** and add 2 mL/well of **Complete Differentiation Medium D** (no wash).

5.8 DAY 8, aspirate **Complete Differentiation Medium D** and add 2 mL/well of **Complete Differentiation Medium E** (no wash).

5.9 DAY 10, aspirate the supernatant, wash once with 2 mL/well of 1× DPBS f, then add 2 mL of Solase and incubate at 37°C, 5% CO_2, and saturated humidity for 3–5 minutes. Centrifuge at 200×g for 5 minutes, remove the supernatant, and resuspend the cells in **Complete Differentiation Medium E.** Passage the cells at a 1:4 ratio and maintain the culture in **Complete Differentiation Medium E**.

5.10 DAY 11, replace the medium with 2 mL/well of Differentiation Complete Medium E.

5.11 DAY13-14, Cells reach full confluence, at which point they are considered P0 hPSC-derived MSCs. **TIPS: P0 hiMSC cells can be cryopreserved.**

5.12 P1 cells can be passaged at a density of 5,000 cells/cm^2.

5.13 P2 hiMSC cells can be used for various scientific research applications.
TIPS: If cryopreservation is required, proceed with the following dissociation steps.

5.14 Aspirate the supernatant, add 2 mL/well of 1× DPBS for a rinse, then add 2 mL of Solase and incubate at 37°C, 5% CO_2, and saturated humidity for 3–5 minutes. Once hiMSCs detach from the culture dish, collect the cells into a 15 mL centrifuge tube and centrifuge at 200×g for 5 minutes.

5.15 Aspirate the supernatant, resuspend hiMSCs in 1 mL/well of serum-free hMSC medium, and take an aliquot for cell counting. Based on the cell count, resuspend the cells in hMSC cryopreservation medium at a density of 2 × 10^6 cells/mL and store in liquid nitrogen.

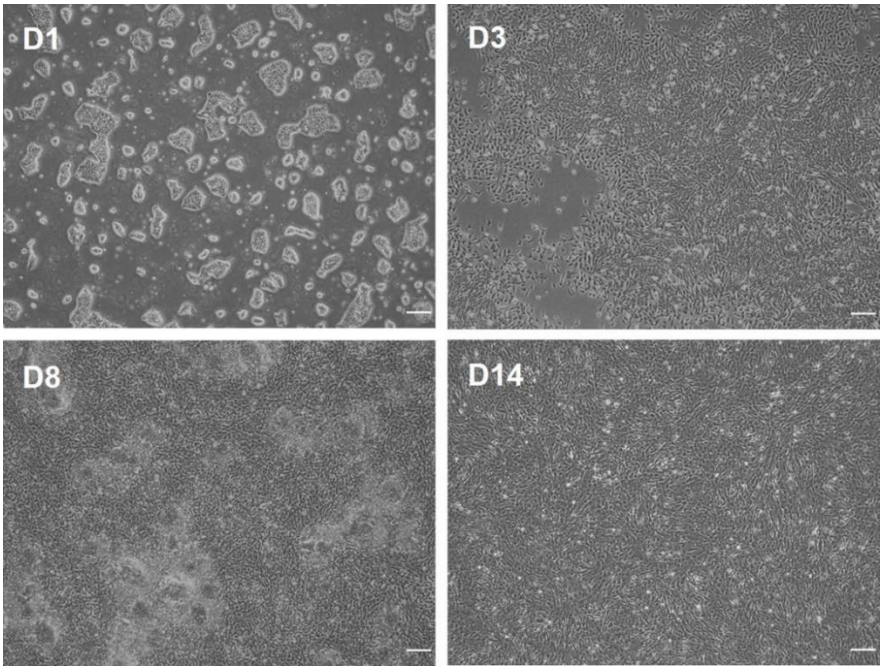


Figure 1: Morphology of hPSC-MSC Differentiation Process.

Images show cell morphology at Days 1, 3, 8, and 14. Scale bar: 200 µm.

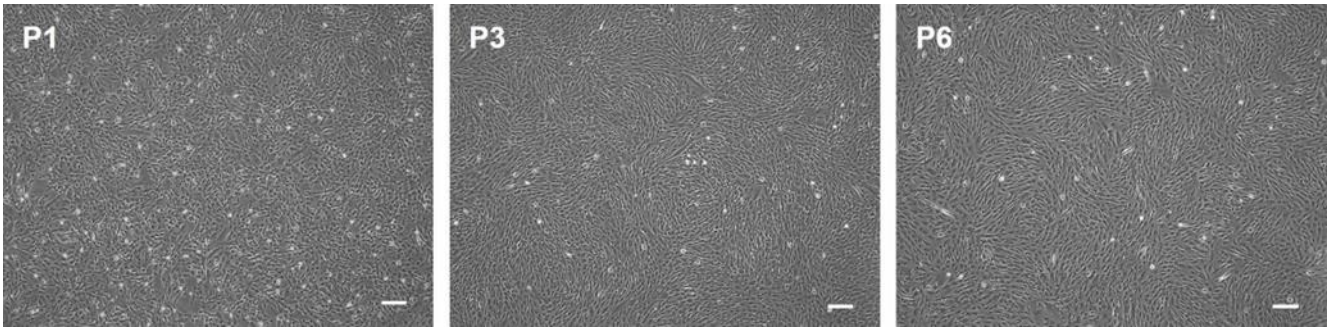


Figure 2: Morphology of hPSC-MSCs During Continuous Culture
Images show P1, P3, and P6 MSCs at 80–85% confluency. Scale bar: 200 µm.