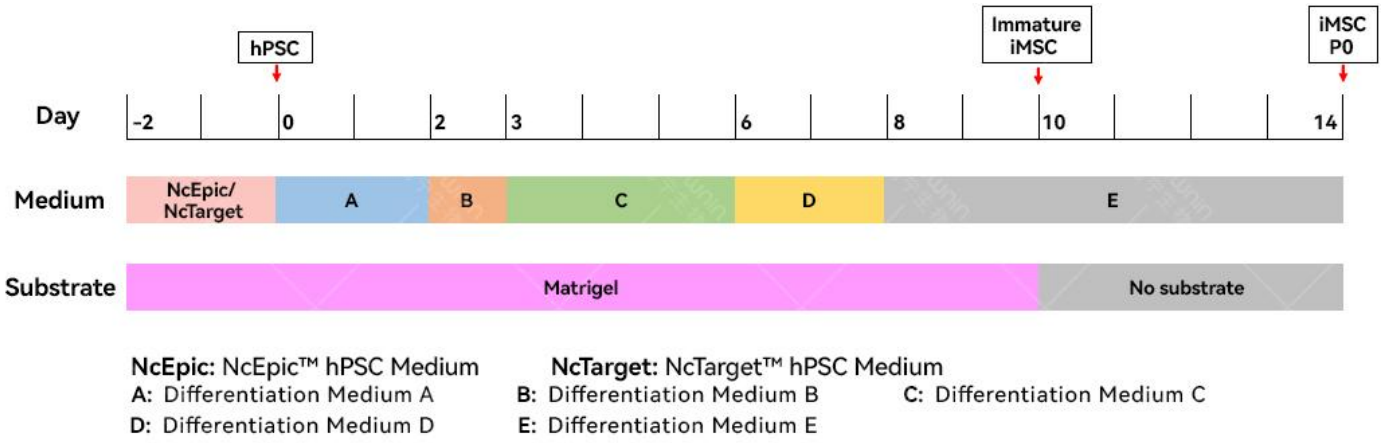
hPSC-MSC Differentiation Kit

Product Manual

Catalog#RP01013 1 Kit

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 ($CD73^+/CD90^+/CD105^+$, $CD14^-/CD34^-/CD45^-/CD79a^-/HLA\text{-}DR^-$), and trilineage differentiation potential (osteogenic, chondrogenic, and adipogenic). hPSC-MSCs are suitable for in vitro studies, drug screening, safety evaluation, and the establishment of disease-related experimental model.



Product Information

Table 1. hPSC-MSC Differentiation Kit Product Description

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

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

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

Reagent and Materials

Table 2. Recommended Reagents & Materials

Reagents & Materials	Brand (e.g.)	Gat.No. (e.g.)
NcEpic™ hPSC Medium	Shownin	SN-01-0010
NcTarget™ hPSC Medium	Shownin	RP01020
hPSC Cryopreservation Medium	Shownin	RP01003
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin (10 mM)	Shownin	RP01008
NcMission™ hMSC Medium V3.0	Shownin	RP02010
hMSC 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

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×)	

hPSC-MSC Differentiation

1. **hPSC Preparation: Refer to the hPSC Medium Instruction Manual for details.**

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

   For a 6-well plate, seed hPSCs at 4 × 10^5 cells/well and maintain plate under standard conditions 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.

2. Day -1, seed hPSCs into a new 6-well plate as described in Step 1 and maintain plate under standard conditions.

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.**

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. 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 2–5 have strict timing requirements for media changes. The recommended schedule is as follows: Step 2: Passage at 11:00 AM; Step 3:30 hours after passage (by 5:00 PM the next Day), replace with Differentiation Complete Medium A; Step 4: On Day 4 at 9:00 AM, replace with Differentiation Complete Medium B.

6. Day 4, Replace the medium with 2 mL/well of **Complete Differentiation Medium C** (no need to wash with DMEM/F12).

7. Day 6, aspirate **Complete Differentiation Medium C** and add 2 mL/well of **Complete Differentiation Medium D** (no need to wash with DMEM/F12).

8. Day 8, aspirate **Complete Differentiation Medium D** and add 2 mL/well of **Complete Differentiation Medium E** (no need to wash with DMEM/F12).

9. Day 10, aspirate the supernatant, wash once with 2 mL/well of 1× DPBS, 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 culture in **Complete Differentiation Medium E**.

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

11. Day13–14, cells reach full confluence, at which point they are considered P0 hPSC-derived MSCs.
    Tips: P0 hiMSC cells can be cryopreserved.

12. P1 cells can be passaged at a density of 5,000 cells/cm².

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

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.

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.

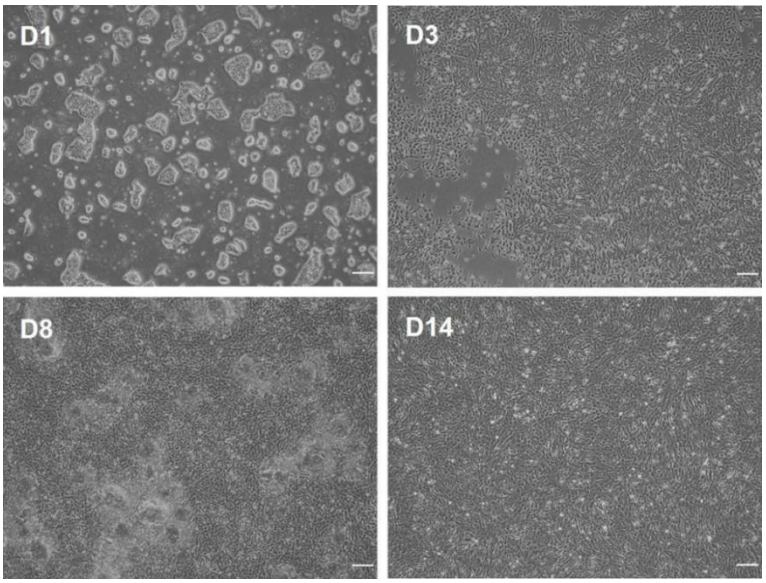


Figure 1: Morphology of hPSC-MSC Differentiation Process.

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

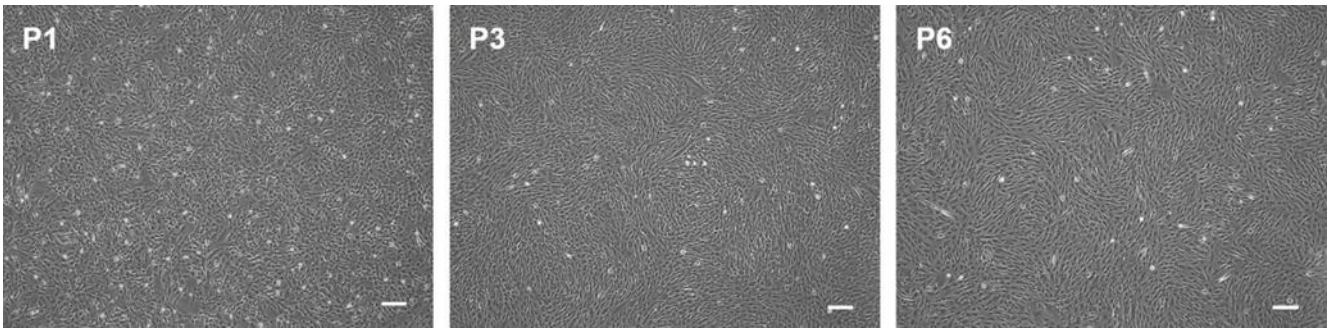


Figure 2: Morphology of hPSC-MSCs During Continuous Culture

Images show P1, P3, and P6 MSCs at 80–85% confluency. Scale bar: 200 µm.