

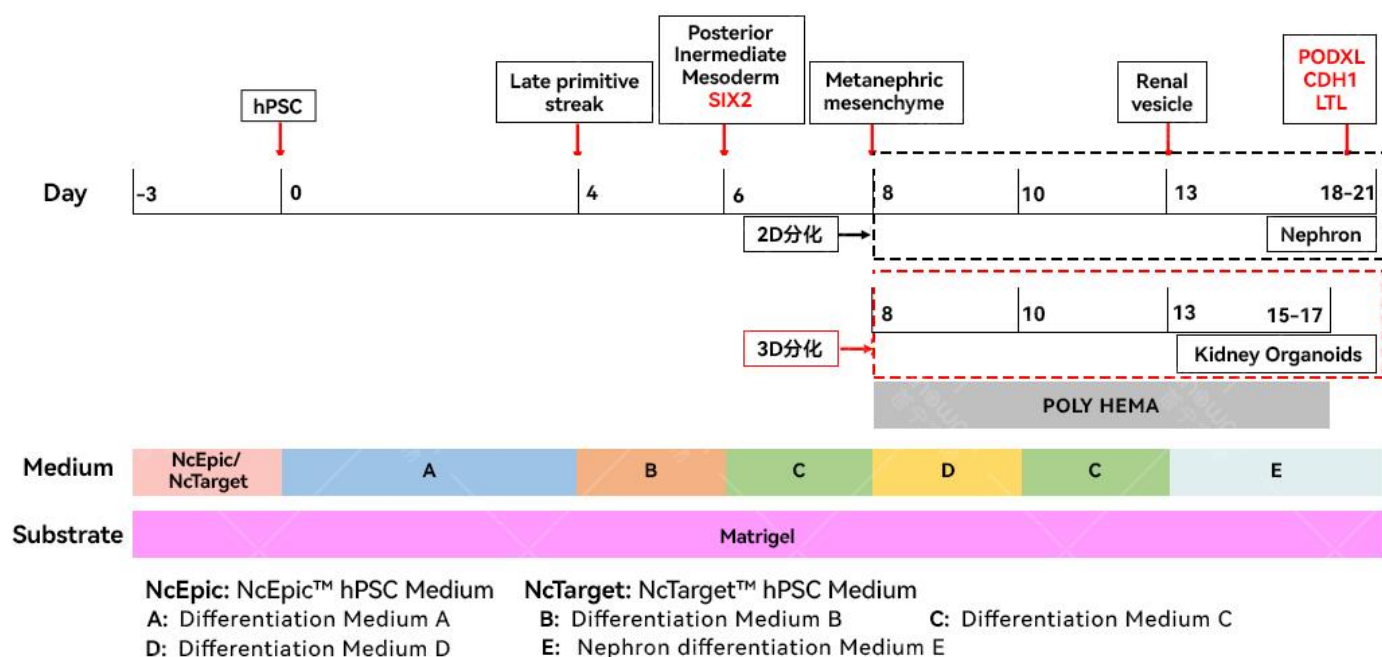
# hPSC-Nephron Differentiation Kit

## Product Manual

### I. Product Introduction

#### 1.1 Product Overview

hPSC-Nephron Cell Differentiation Kit is designed to differentiate human pluripotent stem cells (hPSCs) into renal epithelial cells (RECs), which form nephron-like structures upon maturation. The differentiated nephron-like structures efficiently express specific markers (e.g., CDH1, LTL, and PODXL), making them suitable for various in vitro experiments, drug screening, and safety assessments, as well as cell transplantation studies in disease model animals.



#### 1.2 Product Information

Table 1: hPSC-Nephron Differentiation Product Series

Product Information	Cat. No.	Amount	Storage
hPSC-Nephron Differentiation Kit*	RP01015	1 Kit	Basal Medium 2°C~8°C
hPSC-Nephron Cell Maturation and Differentiation Medium	RP01015-F	1 Kit	Supplements -20°C~-80°C
hPSC-Nephron Cells**	RC01006	3 × 10 <sup>6</sup>	Liquid nitrogen storage

\* Each kit yields 2×10<sup>7</sup> NPCs by Day 8.

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

\*\* Nephron progenitor cells differentiated from hPSC at DAY 7.

### 1.3 Reagents and Materials

**Table 2: Recommended Reagents & Materials & Equipment**

Reagents & Materials	Brand (e.g.)	Cat.No. (e.g.)
NcEpic hPSC Medium	Shownin	RP01001
NcTarget hPSC Medium	Shownin	RP01020
Research-grade hPSC Cell Line	Shownin	RC01001
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin	Shownin	RP01008
hPSC Cryopreservation Medium	Shownin	RP01003
Solase Cell Dissociation Solution	Shownin	RP01021
0.25% Trypsin Solution	Shownin	RP02011
Trypsin Inhibitors	Shownin	RP02012
Corning® Matrigel® Matrix	Corning	354277
DMEM/F12 Medium	Thermo Sci.	11330
DPBS, no calcium, no magnesium	Thermo Sci.	14190144
24-Well Plates	Thermo Sci.	162485
T25 Culture Flasks	Thermo Sci.	156367
1 mL/5 mL/10 mL/25 mL Pipettes	Thermo Sci.	N/A
15 mL/50 mL Centrifuge Tubes	Thermo Sci.	N/A
10 µL/200 µL/1000 µL Pipettes Tips	Rainin.	N/A
Freezing Container	Thermo Sci.	5100-0001
POLY HEMA	Sigma	P3932

## II. hPSC-Nephron Differentiation

### 2.1. Reagent Preparation

**Table 3: Product Description of the hPSC-Nephron Differentiation Kit**

Product Information	Cat.No.	Amount	Storage
The hPSC-Nephron Differentiation Kit * contains:	RP01015	1 Kit	
Nephron Differentiation Supplement A (100 x)	RP01015-A	1 mL	-20°C or -80°C
Nephron Differentiation Supplement B (100 x)	RP01015-B	1mL	-20°C or -80°C
Nephron Differentiation Supplement C (100 x)	RP01015-C	1.5 mL	-20°C or -80°C
Nephron Differentiation Supplement D (100 x)	RP01015-D	1.5 mL	-20°C or -80°C
Nephron Differentiation Medium E	RP01015-E	500 mL	2°C~8°C

\* Each kit yields  $2 \times 10^7$  NPCs on DAY 8.

\* Each kit supports 2D differentiation in one 24-well plate or 3D differentiation in 12 wells (of a 24-well plate).

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

2.1.1. Thaw Nephron Differentiation Supplement A, B, C, and D at 4°C, do not **thaw at 37°C**.

2.1.2. In a biosafety cabinet, prepare **Complete Differentiation Medium A/B/C/D** with reference to Table 4.

2.1.3. Prepared media should be **used immediately** or stored at 4°C for up to 2 weeks.

**TIPS: Aliquot Nephron Differentiation Supplement A/B/C/D for freezing if needed. Avoid more than 2 freeze-thaw cycles.**

**Table 4: Reagent Preparation Instructions for the hPSC- Nephron Differentiation Kit**

Medium Type	Components	Final Concentration
<b>Complete Differentiation Medium A/B/C/D (1 x)</b>	Nephron Differentiation Supplement a (100 x) / b (100 x) / c (100 x) / d (100 x)	1x
	Nephron Differentiation Medium E	

### 2.2 hPSC-Nephron 2D Differentiation

2.2.1. **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>

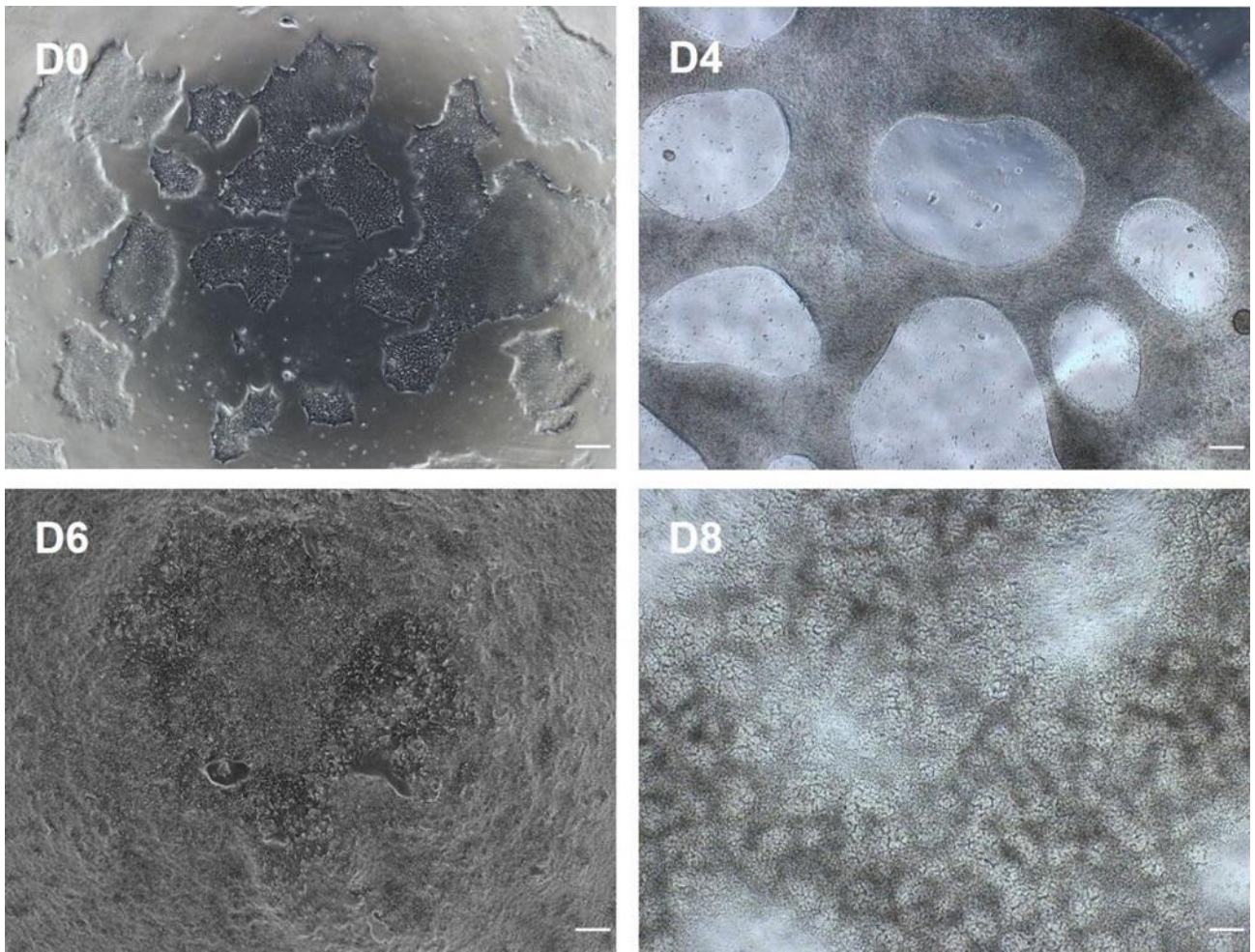
2.2.2. **Day -3**, taking the operation of a 24-well plate as an example, the seeding density of hPSCs is  $3 - 5 \times 10^4$  cells/well, with medium change every day.

**TIPS: The seeding density of hPSCs is  $5 \times 10^4$  cells/cm<sup>2</sup>, using 200  $\mu$ L/cm<sup>2</sup> of hPSC Complete Medium (NcEpic or NcTarget). It is recommended to culture hPSC cells for at least 5 passages after thawing before directing differentiation.**

- 2.2.3. **DAY 0**, when the confluence of hPSC cells reaches 50%, aspirate the hPSC Complete Medium (NcEpic or NcTarget), wash the cells once with 500  $\mu$ L DPBS (without calcium and magnesium), and then add 0.5 mL/well Complete Differentiation Medium A. Change the medium daily and culture for 4 days (DAY 0–DAY 4).  
**TIPS: The time to reach 50% confluence may vary slightly due to differences in initial seeding density and cell condition. The differentiation start time can be adjusted according to when 50% confluence is achieved.**
- 2.2.4. **Day 4**, aspirate **Complete Differentiation Medium A**, add 1mL/well **Complete Differentiation Medium B**. Change the medium every day, and culture for 2 days (Day 4 - Day 6).  
**TIPS: After culturing with Complete Differentiation Medium A for 4 days, cell retraction occurs. Both overly loose and overly dense cell retraction may affect the subsequent differentiation efficiency (Figure 1-B).**
- 2.2.5. **Day 6**, aspirate **Complete Differentiation Medium B**, add 1mL/well **Complete Differentiation Medium C**. Change the medium every day, and culture for 2 days (Day 6 - Day 8).
- 2.2.6. **Day 8**, aspirate **Complete Differentiation Medium C**, add 1mL/well **Complete Differentiation Medium D**. Change the medium every day, and culture for 2 days (Day 8 - Day 10).
- 2.2.7. **Day 10**, aspirate **Complete Differentiation Medium D**, add 1mL/well **Complete Differentiation Medium C**. Change the medium every day, and culture for 3 days (Day 10 - Day 13).
- 2.2.8. **DAY 13**, aspirate **Complete Differentiation Medium C**, and add 1 mL/well **Nephron Differentiation Medium E**. Change the medium daily, and mature nephron epithelial cell structures can be obtained after approximately 5 -7 days (DAY 13–DAY 18/21).

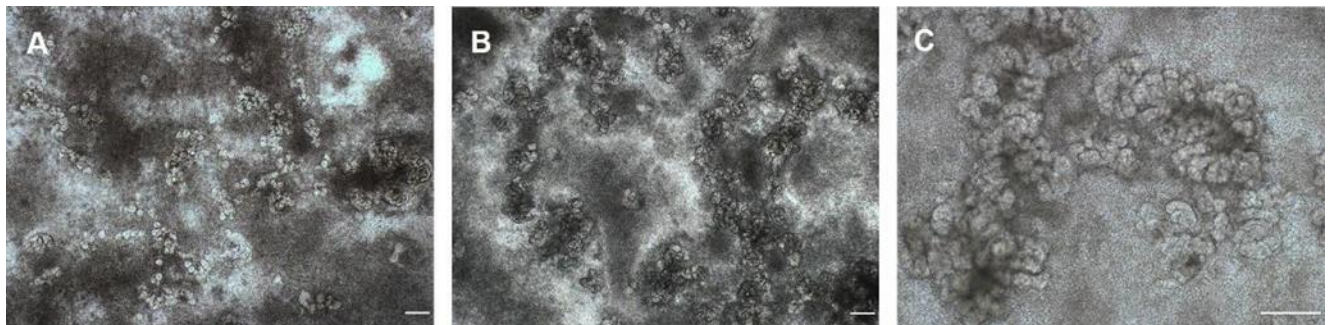
### 2.3 hPSC-Nephron 3D Differentiation

- 2.3.1. The culture and preparation of hPSCs, as well as the differentiation procedures from DAY 0 to DAY 8, are detailed in Sections **2.2.1–2.2.6**.
- 2.3.2. **DAY 8**, transfer to 3D culture: Prepare 6 mL of **the complete differentiation medium D** in advance, add **Blebbistatin** at a ratio of 1:1000. Add 500  $\mu$ L of **0.25% trypsin digestion solution** to one well of a 24-well plate. After incubation at 37 °C for 3 minutes, add 500  $\mu$ L of **trypsin inhibitor** to terminate the digestion. Use a 1 mL pipette to gently pipette the cells. Then transfer the cell suspension to a 1.5 mL centrifuge tube, centrifuge instantaneously in a palm centrifuge for 5 - 10 seconds, and aspirate and discard the supernatant. Add **the complete differentiation medium D containing Blebbistatin** to resuspend the cells and transfer them to a **POLY HEMA-coated T25 culture flask**. Place it on a three-dimensional shaker and culture at a rotation speed of 15 rpm. Change the medium every day (from Day 8 to Day 10).  
**TIPS: After 24 hours, aspirate Blebbistatin and replace it with fresh medium. Blebbistatin after 24 hours and add fresh medium.**
- 2.3.3. **Day 10**, aspirate **Complete Differentiation Medium D**, add 6mL/well **Complete Differentiation Medium C**. Change the medium every day, and culture for 3 days (Day 10 - Day 13).
- 2.3.4. **DAY 13**, aspirate **Complete Differentiation Medium C**, add 5mL/well **Nephron differentiation Medium E**. Change the medium every day, and structurally distinct Kidney Organoids can be obtained in approximately 2–4 days (DAY 13–DAY 15/17).



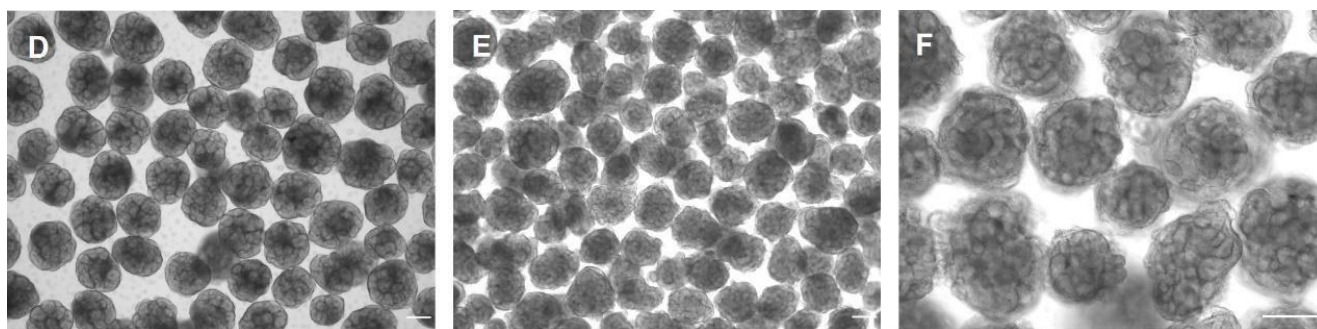
**Morphology of hPSC-Kidney Organoid Differentiation (2D)**

Images show Day 0, 4, 6, and 8 morphologies. Scale bar: 200  $\mu$ m.



**Morphology of Mature 2D Cultures**

Day 14 (A) and Day 20 (B, C) structures. Scale bar: 200  $\mu$ m.



Morphology of 3D Kidney Organoids

Day 13 (D) and Day 17 (E, F) structures. Scale bar: 200 μm.

### III. hPSC-Nephron Cell Thawing and Further Differentiation

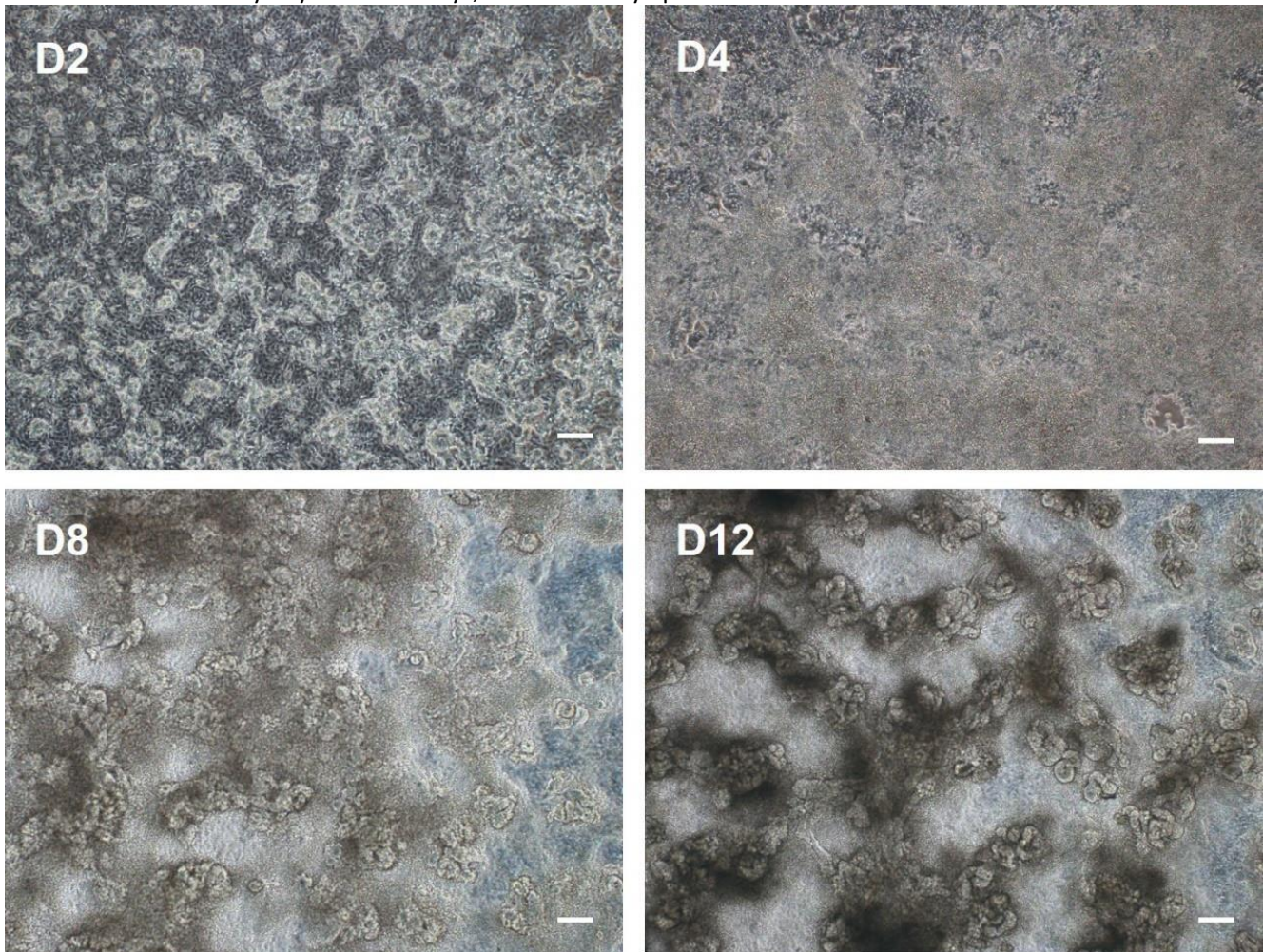
#### 3.1. Reagent Preparation

Table 5: hPSC-Nephron Cell Culture System

Product Information	Cat.No.	Amount	Storage
hPSC-Nephron cells	RC01006	$3 \times 10^6$	Liquid nitrogen storage
hPSC-Nephron Cell Differentiation Medium	RP01015-F	1 Kit	
Nephron Differentiation Supplement C (100 x)	RP01015-C	1.5 mL	-20°C or -80°C
Nephron Differentiation Supplement D (100 x)	RP01015-D	1.5 mL	-20°C or -80°C
Nephron Differentiation Medium E	RP01015-E	500 mL	2°C~8°C

- 3.1.1. Thaw **Nephron differentiation Supplement C and D** at 4°C, **do not thaw at 37°C**.
  - 3.1.2. In a biosafety cabinet, prepare **Complete Differentiation Medium C/D (1x)** with reference to Table 4.
  - 3.1.3. Prepared media should be **used immediately** or stored at 4°C for up to 2 weeks.  
**TIPS: Aliquot Nephron Differentiation Supplement C/D for freezing if needed. Avoid more than 2 freeze-thaw cycles.**
- #### 3.2 hPSC-Nephron Cell Thawing and Further Differentiation-2D
- 3.2.1. Preheat the water bath to 37°C. Place the Matrigel-coated 24-well plate in a biosafety cabinet for about 30 minutes to reach room temperature. Take 1 mL of **Complete Differentiation Medium C**, add **Blebbistatin** at a 1:2000 ratio (final concentration: 5 μM), and allow it to reach room temperature.
  - 3.2.2. Take out one tube of frozen **hPSC-Nephron cells** from the liquid nitrogen tank and transfer it on dry ice to the cell culture room. Immediately place the vial in a 37°C water bath, gently swirling it. Thaw within 1 minute and remove it as soon as ice crystals in the cell suspension are nearly dissolved.
  - 3.2.3. Wipe the surface of the cryovial with 75% ethanol wipes and transfer it to a biosafety cabinet; transfer the cell suspension to a pre-prepared 15 mL centrifuge tube, pipette 8 mL of **Nephron differentiation Medium E**, and add to the frozen cell suspension drop by drop while gently swirling to mix the cells. Centrifuge at 150 xg for 5 minutes.
  - 3.2.4. Aspirate the supernatant. Add 1 mL of pre-warmed **Complete Differentiation Medium C (containing Blebbistatin)** to resuspend the cells. Avoid excessive pipetting. Transfer the cell suspension into one well of a Matrigel-coated 24-well plate. Place the plate in a 37°C, 5% CO<sub>2</sub> incubator with saturated humidity. Gently shake the plate in a horizontal "X" direction 3 times to ensure even distribution. Continue culture.
  - 3.2.5. **Day 2**, aspirate **Complete Differentiation Medium C**, add 1mL/well **Complete Differentiation Medium D**. Change the medium every day, and culture for 2 days (Day 2 - Day 4).
  - 3.2.6. **DAY 4**, aspirate **Complete Differentiation Medium D**, add 1mL/well **Complete Differentiation Medium C**. Change the medium every day, and culture for 3 days (DAY4-DAY 7).
  - 3.2.7. **DAY 7**, aspirate **Complete Differentiation Medium C**, add 1mL / well **Nephron differentiation Medium E**. Change

the medium every day. After 5-7 days, mature kidney epithelial cell structures can be obtained.



**Morphology of hPSC-Derived Nephron Cells (2D Differentiation, Days 2–12)**  
Scale bar: 200  $\mu$ m.

### 3.3 hPSC-Nephron Cell Thawing and Further Differentiation-3D

- 3.3.1. Add 6 mL of **Complete Differentiation Medium C** and **Blebbistatin** at a 1:1000 ratio. Follow the steps in [sections 3.2.3 - 3.2.4](#) for cell thawing and collect the cell pellet.
- 3.3.2. Resuspend the cells in **Complete Differentiation Medium C (containing Blebbistatin)** and transfer them to a POLY HEMA-coated T25 culture flask. Place the flask on a 3D shaker at 15 rpm for cultivation.
- 3.3.3. **DAY1**, aspirate **the Complete Differentiation Medium C**, add 6 mL/well **Complete Differentiation Medium D**. Change the medium every day, and culture for 2 days.
- 3.3.4. **DAY3**, aspirate **the Complete Differentiation Medium D**, add 6 mL/well **Complete Differentiation Medium C**. Change the medium every day, and culture for 3 days.
- 3.3.5. **DAY 6**, aspirate **Complete Differentiation Medium C**, add 5 mL/well **Nephron differentiation Medium E**. Change the medium every day. Kidney Organoids with obvious structures can be obtained approximately 2 - 4 days later.