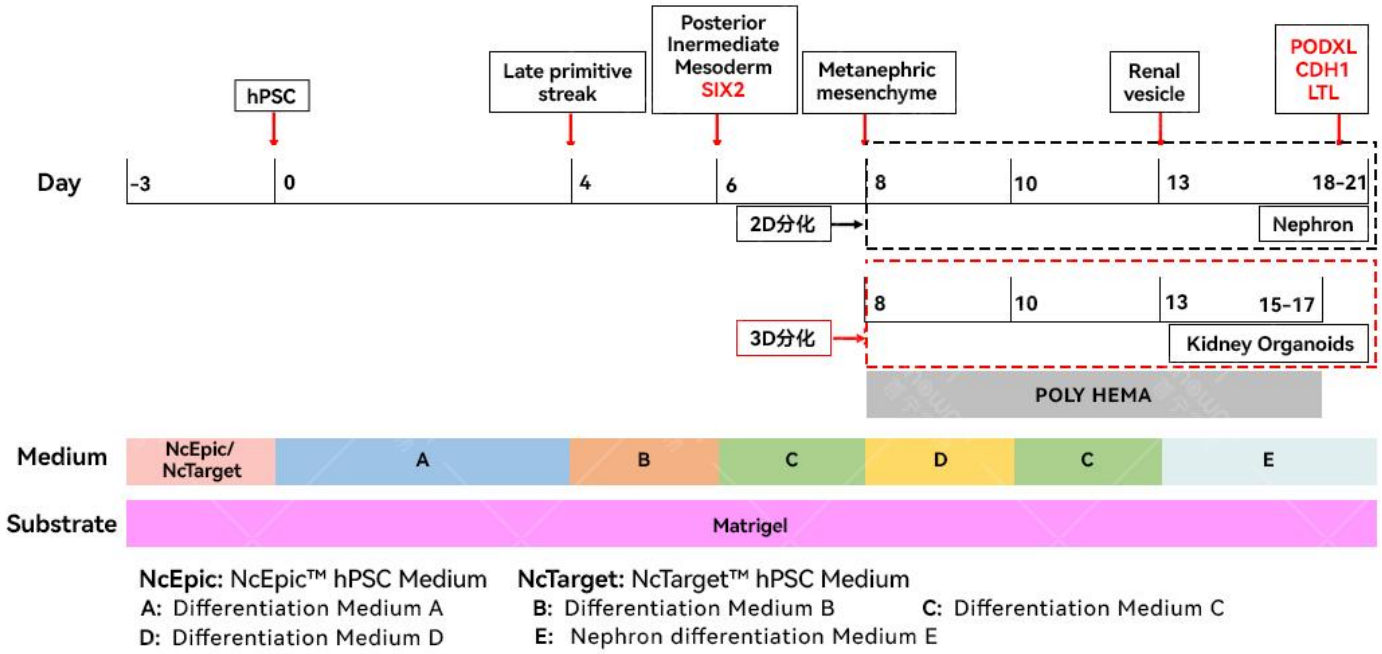
hPSC-Nephron Differentiation Kit

Product Manual

Catalog#RP01015 1 Kit

Product Introduction

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 the establishment of disease-related experimental model.



Product Information

Table 1. hPSC-Nephron Differentiation Kit Product System

Product	Cat. No.	Amount	Storage
hPSC-Nephron Differentiation Kit*	RP01015	1 Kit	Basal Medium 2–8 °C Supplements -80 °C or-20 °C
hPSC-Nephron Cell Maturation and Differentiation Medium	RP01015-F	1 Kit	

*** Each kit yields 2 × 10^7 NPCs by Day 8.**

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

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	-80 °C or-20 °C
Nephron Differentiation Supplement B (100 x)	RP01015-B	1 mL	
Nephron Differentiation Supplement C (100 x)	RP01015-C	1.5 mL	
Nephron Differentiation Supplement D (100 x)	RP01015-D	1.5 mL	
Nephron Differentiation Medium E	RP01015-E	500 mL	2–8 °C

*** Each kit yields 2 × 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 2–8 °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
Complete Differentiation Medium A/B/C/D (1×)	Nephron Differentiation Supplement a (100×) / b (100×) / c (100×) / d (100×)	1×
	Nephron Differentiation Medium E	

2.2 hPSC-Nephron 2D Differentiation

2.2.1. **hPSC Preparation: Refer to the hPSC culture Medium Instruction Manual for details. (https://www.shownin.com/download/8.html?page=1, Operation Manual)**

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

Tips: The seeding density of hPSCs is 5 × 10^4 cells/cm², using 200 µL/cm² of hPSC Complete Medium (NcEpic or NcTarget). It is recommended for at least 5 cell passages after thawing before directing differentiation.

2.2.3. **Day 0,** when the confluence of hPSCs reaches 50%, aspirate the hPSC Complete Medium (NcEpic or NcTarget), wash the cells once with 500 µL DPBS (without calcium and magnesium), and then add 0.5 mL/well **Complete Differentiation Medium A**. Change the medium daily and maintain plate under standard conditions 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 1 mL/well **Complete Differentiation Medium B**. Change the medium every Day, and maintain plate under standard conditions for 2 Days (Day 4–Day 6).

Tips: The cells will exhibit retraction after 4 days. 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 maintain plate under standard conditions 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 maintain plate under standard conditions 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 maintain plate under standard conditions 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

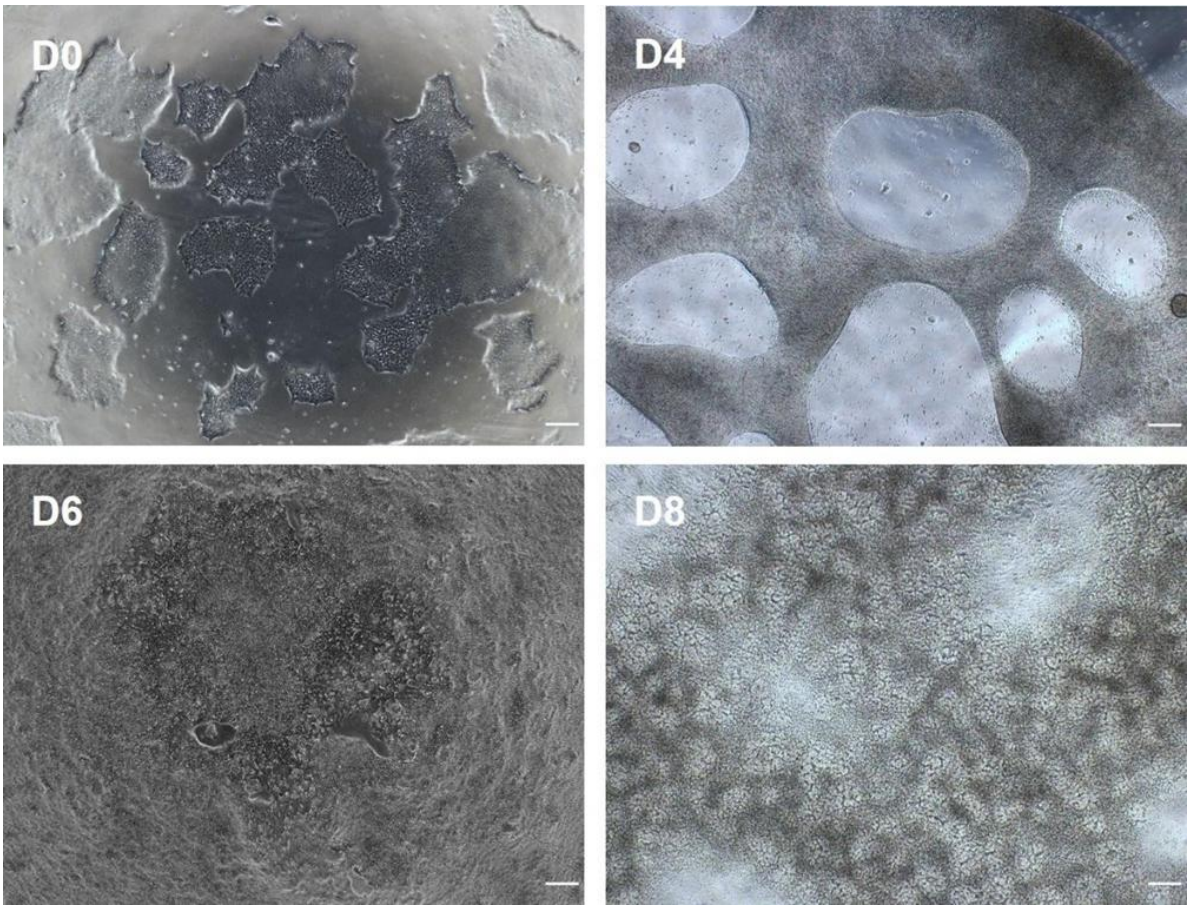
2.3.1. The preparation of hPSCs, as well as the differentiation procedures from Day 0 to Day 8, are detailed in Sections **2.2.1–2.2.6.**

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 µL of **0.25% trypsin digestion solution** to one well of a 24-well plate. After incubation at 37 °C for 3 minutes, add 500 µL of **trypsin inhibito**r 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 the flask on a three-dimensional shaker with a rotation speed of 15 rpm and maintain culture. 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.

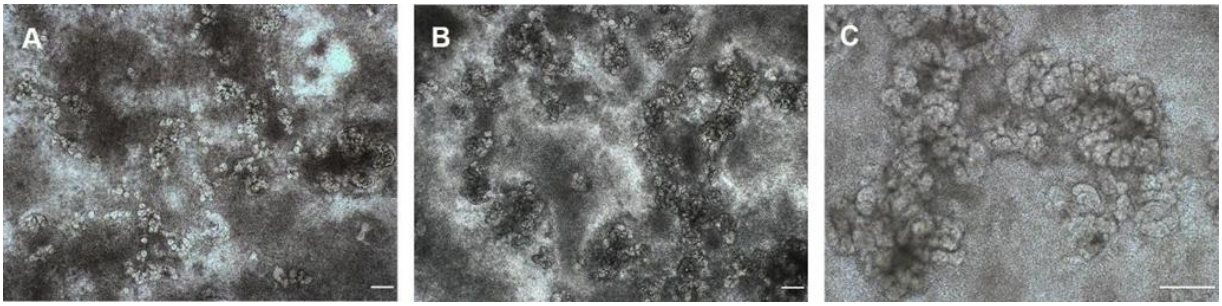
2.3.3. **Day 10,** aspirate **Complete Differentiation Medium D,** add 6 mL/well **Complete Differentiation Medium C**. Change the medium every Day, and maintain culture for 3 Days (Day 10–Day 13).

2.3.4. **Day 13**, aspirate **Complete Differentiation Medium C,** add 5 mL/well **Nephron differentiation Medium E.** Change the medium every Day, and structurally distinct Kidney Organoids can be obtained in approximately 2–4 Days (Day13–Day15/17)



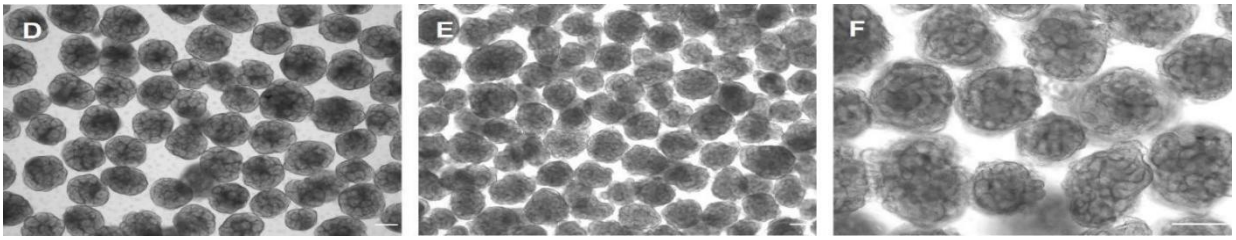
Morphology of hPSC-Kidney Organoid Differentiation (2D)

Images show Day 0, 4, 6, and 8 morphologies. Scale bar: 200 µm.



Morphology of Mature 2D Cultures

Day 14 (A) and Day 20 (B, C) structures. Scale bar: 200 µm.



Morphology of 3D Kidney Organoids

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

hPSC-Nephron Cell Thawing and Further Differentiation

3.1 Reagent Preparation

Table 5. hPSC-Nephron Cell Differentiation Medium Product System

Product Information	Cat.No.	Amount	Storage
hPSC-Nephron Cell Differentiation Medium*Contains:	**RP01015-F**	**1 Kit**	
Nephron Differentiation Supplement C (100×)	RP01015-C	1.5 mL	-80 °C or -20 °C
Nephron Differentiation Supplement D (100×)	RP01015-D	1.5 mL	-80 °C or -20 °C
Nephron Differentiation Medium E	RP01015-E	500 mL	2–8 °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 (1×)** 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.

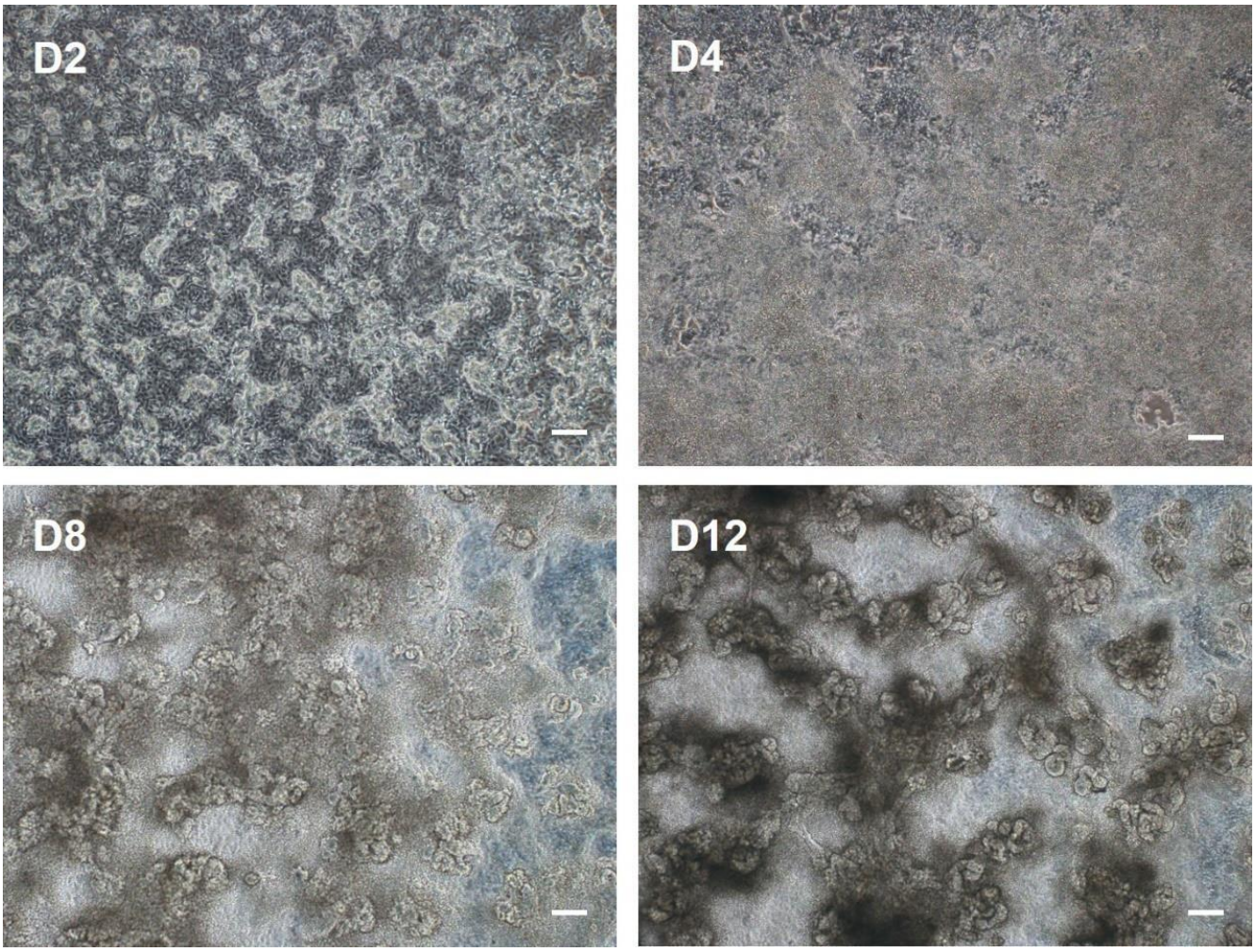
3.2.3. Wipe the surface of the cryovial with 75% ethanol wipes and transfer it to a biosafety cabinet; transfer the cell suspension to a pre-prepared 15 mL centrifuge tube, pipette 8 mL of **Nephron differentiation Medium E**, and add to the frozen cell suspension drop by drop while gently swirling to mix the cells. Centrifuge at 150 × g 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_2 incubator with saturated humidity. Gently shake the plate in a horizontal "X" direction 3 times to ensure even distribution. Maintain culture.

3.2.5. **Day 2,** aspirate **Complete Differentiation Medium C,** add 1mL/well **Complete Differentiation Medium D**. Change the medium every Day, and maintain 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 maintain culture for 3 Days (Day4–Day 7).

3.2.7. **Day 7**, aspirate **Complete Differentiation Medium C,** add 1 mL/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 µm.

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 maintain 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 maintain culture for 3 Days.

3.3.5. **Day 6**, aspirat **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.