Research-Grade hiPSC Line
Product Manual

I. Product Introduction

Research-grade human induced pluripotent stem cell (hiPSC) lines are derived from human somatic cells using a non-integrating reprogramming method. These cells are cultured in a feeder-free, chemically defined hPSC medium (NcEpic or NcTarget) and exhibit stable proliferation. They share morphological characteristics, cell types, and gene expression profiles with human embryonic stem cells (hESCs). Quality control ensures normal chromosomal karyotypes, absence of exogenous gene integration, and trilineage differentiation potential.

II. Product Information

Table 1: Research-Grade hiPSC Cell Line Product Description

Product Information	Cat.No.	Amount	Description
Research-Grade hiPSC Cells - Male	RC01001-A	＞2×10^6 /tube	36 years old, blood source.
Research-Grade hiPSC Cells - Female	RC01001-B		0 years old, umbilical cord source.
hiPSC-EGFP	RC01004	>2×10^6/tube	Random insertion of EGFP gene fragment, whole-cell green fluorescence; cryopreserved in liquid nitrogen.
hiPSC-Luc-GFP	RC01010	>2×10^6/tube	EGFP gene fragment with nuclear green fluorescence and luciferase expression; cryopreserved in liquid nitrogen.
hiPSC-mCherry	RC01016	>2×10^6/tube	mCherry gene fragment inserted at ROSA26 locus, whole-cell red fluorescence; cryopreserved in liquid nitrogen.

Research-Grade hiPSC Cell Lines Information	
Induction Method	Non-integrated reprogramming technology
Passage Number	p10-20
Surface Marker Detection	Flow cytometry: SSEA4+ / TRA-1-81+
Pluripotency Gene Detection	qRT-PCR: OCT4 + / NANOG +
Exogenous Gene Detection	No exogenous gene insertion
Genomic Stability	Normal chromosomal karyotype
Differentiation Potential	Stable trilineage differentiation in teratoma assay
Donor Cell Infectious Disease Screening	Negative for HBV, HCV, HIV, and syphilis
Microbial Testing	Negative for bacteria, fungi, and mycoplasma
Endotoxin Testing	<2.5 EU/mL
Shipping Condition	Dry ice
Storage Condition	-196°C

*** This product is for research use only and is not intended for diagnostic or therapeutic applications.**

III. Reagents and Materials

Table 2: Recommended Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat.No.(e.g.)
NcEpic™ hPSC Medium	Shownin	RP01001
NcTarget™ hPSC Medium	Shownin	RP01020
Vitronectin	Shownin	RP01002
hPSC Dissociation Buffer	Shownin	RP01007
Blebbistatin (10 mM)	Shownin	RP01008
hPSC Cryopreservation Medium	Shownin	RP01003
DMEM/F12 Medium	Thermo Sci.	11330
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.	1535050

IV. Reagent Preparation

(i) Preparation of hPSC Complete Medium (NcEpic™ or NcTarget™) (500 mL)

1.1. Preparation of NcEpic™ Pluripotent Stem Cell Complete Medium (500 mL)

1.1.1. Thaw NcEpic™ Supplement at 4℃. Do not thaw at 37℃.

1.1.2. In a biosafety cabinet, use a sterile pipette to mix the following components to prepare 500 mL of complete medium.

NcEpic™ Basal Medium: 496 mL

NcEpic™ Supplement: 4 mL

1.1.3. The complete medium can be stored at 4℃ and used within 2 weeks.

TIPS: The NcEpic™ Supplement can be aliquoted and frozen for future use. For example, aliquot 0.8 mL×5 tubes. Thaw 0.8 mL Supplement and mix with 99.2 mL Basal Medium to prepare 100 mL of complete medium. The Supplement should not undergo more than 2 freeze-thaw cycles.

1.2. Preparation of NcTarget™ hPSC Complete Medium (500 mL)

1.2.1. Thaw NcTarget™ Supplement A/B at 4℃. Do not thaw at 37℃.

1.2.2. Refer to Table 3 to mix the following components in a biosafety cabinet using a sterile pipette to prepare the complete medium. The complete medium can be stored at 4℃ and used within 2 weeks.

Table 3: Preparation of NcTarget™ Complete Medium

Component	500 mL Complete Medium	100 mL Complete Medium	50 mL Complete Medium
NcTarget™ Basal Medium	400 mL	80 mL	40 mL
NcTarget™ Supplement-A	20 mL	4 mL	2 mL
NcTarget™ Supplement-B	80 mL	16 mL	8 mL

***The NcTarget™ Supplement A/B can be aliquoted and frozen for future use. Refer to Table 3 for preparation instructions. The NcTarget™ Supplement A/B should not undergo more than 2 freeze-thaw cycles.**

(ii) Coating of Culture Plates with Vitronectin (Using 6-Well Plate as an Example, Applicable to Other Culture Vessels**)**

2.1. Coat the culture dish with Vitronectin under sterile conditions.

2.2. Thaw Vitronectin at room temperature (15 - 25℃).

TIPS: Thawed Vitronectin can be stored at 4℃ for up to 2 weeks. It can also be aliquoted and stored at -20℃ or -80℃ for use within the expiration date. Avoid repeated freeze-thaw cycles.

2.3. Aliquot Vitronectin: Recommended coating concentration is 1 μg/cm². For a 6-well plate with a well area of 10 cm²/well, 60 μg of Vitronectin is required per plate, i.e., 120 μL (500 μg/mL). It is recommended to aliquot Vitronectin into 120 μL (60 μg) tubes and store at -20℃ or -80℃. Each tube can coat one 6-well plate.

2.4. Take one tube of Vitronectin (120 μL, 60 μg) and dilute it with 9 mL of DMEM/F12. Mix gently without creating bubbles.

2.5. Dispense 1.5 mL/well into a 6-well plate. Gently swirl to ensure even distribution of the diluted Vitronectin solution across the well surface.

2.6. Allow the plate to sit at room temperature (15 - 25℃) for at least 1 hour before use. When ready to use, tilt the plate and remove the coating solution using a pipette or tip. Ensure the coated plate surface is free of scratches and does not require additional washing.

TIPS: If not used immediately, seal the plate to prevent evaporation of the Vitronectin solution. Coated plates can be stored at 4℃ for up to 1 week. Before use, allow the plate to warm to room temperature (15 - 25℃) for 10 - 30 minutes. If the Vitronectin solution evaporates, causing the plate surface to dry, it may severely affect the attachment of hESCs and hiPSCs.

(iii) Coating of Culture Plates with Matrigel (Using 6-Well Plate as an Example, Applicable to Other Culture Vessels**)**

A. Aliquoting Matrigel

1. Check the concentration of the received Matrigel batch. Calculate the aliquot volume and quantity based on the recommended coating concentration and coating area.

Example: For hPSC culture, the recommended Matrigel coating concentration is 0.013 mg/cm², i.e., 0.75 mg per 6-well plate. If the Matrigel concentration is 11.3 mg/mL (10 mL), aliquot 3 mg/tube (enough to coat four 6-well plates). Aliquot volume per tube = 3 mg / 11.3 mg/mL = 0.265 mL. Number of aliquots = 10 mL / 0.265 mL = 37.74.

2. Prepare 38 sterile 1.5 mL EP tubes. Label with Matrigel batch number, concentration, date, and operator ID. Pre-cool 1000 μL sterile tips and EP tube racks at -20℃ for 1 hour.

TIPS: For Matrigel with catalog number 354277 (hESC-Qualified Matrigel), the protein concentration is not indicated in the instructions. Instead, the Dilution Factor is provided. If the recommended Dilution Factor for a batch is 238 μL,

it means 238 µL can coat 4 six-well plates. Number of aliquots = 5 mL / 0.238 = 21.01.

3. Thaw Matrigel at 4℃ overnight. Begin aliquoting when Matrigel is completely thawed.

TIPS: Matrigel remains liquid only at 4℃. If the refrigerator temperature fluctuates frequently, Matrigel may not remain liquid.

4. Prepare an ice box filled with crushed ice. Place the thawed Matrigel, pre-cooled 1.5 mL EP tubes, EP tube racks, and 1000 µL tips on the ice box.
5. Mix Matrigel and aliquot into the 1.5 mL EP tubes. Keep the tubes on ice. Replace the tip if it becomes clogged, as this may affect the aliquot volume.
6. Store the aliquoted Matrigel at -20℃.

B. Coating Plates

1. Prepare 36 mL of cold DMEM/F12 in a 50 mL centrifuge tube. Label 4 six-well plates with Matrigel batch number, date, and operator ID.
2. Pre-cool 1000 µL sterile tips at -20℃ for 1 hour. Thaw one frozen Matrigel aliquot (3 mg) at 4℃ until completely thawed.
3. Prepare an ice box filled with crushed ice. Place the thawed Matrigel and pre-cooled 1000 µL tips on the ice box.
4. Use a pre-cooled tip to add 1 mL of cold DMEM/F12 to the thawed Matrigel (3 mg). Mix by pipetting up and down to dissolve and mix thoroughly.
5. Add the mixed Matrigel to the remaining DMEM/F12 in the centrifuge tube. Use a 10 mL pipette to mix thoroughly by pipetting up and down.
6. Dispense 1.5 mL/well into 4 six-well plates. Gently swirl to ensure even distribution.
7. Allow the plates to sit at room temperature for 1 hour before use, or store at 4℃ overnight. Use within 2 weeks.

V. Thawing hPSCs (Using 6-Well Plate as an Example, Applicable to Other Culture Vessels)

1. Preheat the water bath to 37°C.
2. Place the Matrigel-coated 6-well plate in the biosafety cabinet for about 1 hour to warm to room temperature (15~30℃).
3. Prepare 4 mL of hPSC complete medium (NcEpic or NcTarget). Add 1 µL of Blebbistatin (10 mM) at a 1:4000 ratio. Allow the medium to reach room temperature (15~30℃).

TIPS: Do not pre-warm the medium in a 37℃ water bath.

4. Thaw one vial of cryopreserved cells in a 37℃ water bath by gently shaking. Thaw within 1 minute. Remove when the ice crystals are almost completely dissolved (remaining ice crystals should be the size of a mung bean).
5. Wipe the cryovial surface with 75% alcohol and transfer to the biosafety cabinet. Transfer the cell suspension to a 15 mL centrifuge tube. Add 10 mL of DMEM/F12 dropwise while gently shaking the tube. Centrifuge at 160 xg for 5 minutes.
6. Discard the supernatant. Add 4 mL of pre-warmed Blebbistatin + hPSC complete medium (NcEpic™ or NcTarget™) to resuspend the cells. Avoid pipetting.
7. Remove the Matrigel coating solution from 2 wells of the 6-well plate. Inoculate the resuspended cells at 2 mL/well into the 2 wells.
8. Gently swirl the plate three times horizontally. Place in the incubator (37°C, 5% CO_2, saturated humidity). Swirl again three times horizontally.Continuous culture for 18~24 hours.
9. Replace with fresh hPSC complete medium (NcEpic™ or NcTarget™) after 18~24 hours. Change the medium

daily thereafter.

Table 4: Recommended Reagent Usage for hPSC Passage & Culture

Culture Vessel	Growth Area	DPBS (mL)	EDTA Solution	hPSC Complete Medium*
6-well Plate	9.6 cm^2 /well	2 mL/well	2 mL/well	2 mL/well
12-well plate	4.5 cm^2 /well	1 mL/well	1 mL/well	1 mL/well
24-well plate	2 cm^2 /well	0.5 mL/well	0.5 mL/well	0.5 mL/well
35mm culture dish	8 cm^2	2 mL	2 mL	2 mL
60mm culture dish	21 cm^2	4 mL	4 mL	4 mL
100mm culture dish	55 cm^2	10 mL	10 mL	10 mL

*** During routine hPSC culture, when cell confluency exceeds 50%, it is recommended to add an extra 50% medium during medium change. For example, add 3 mL of medium per well in a 6-well plate.**

VI. Passage of hPSCs (Using 6-Well Plate and EDTA Dissociation as an Example, Applicable to Other Culture Vessels)

1. Passage Timing:
   1.1. When cell confluency reaches approximately 85% (Figure 1), subculture every 4 days. Even if the colonies are small and confluency is low, do not culture for more than 5 days.
   1.2. If cell confluency is low but the stem cell colonies are too large, with poor growth in the center.
   1.3. Passage hiPSCs when any of the above conditions are met.

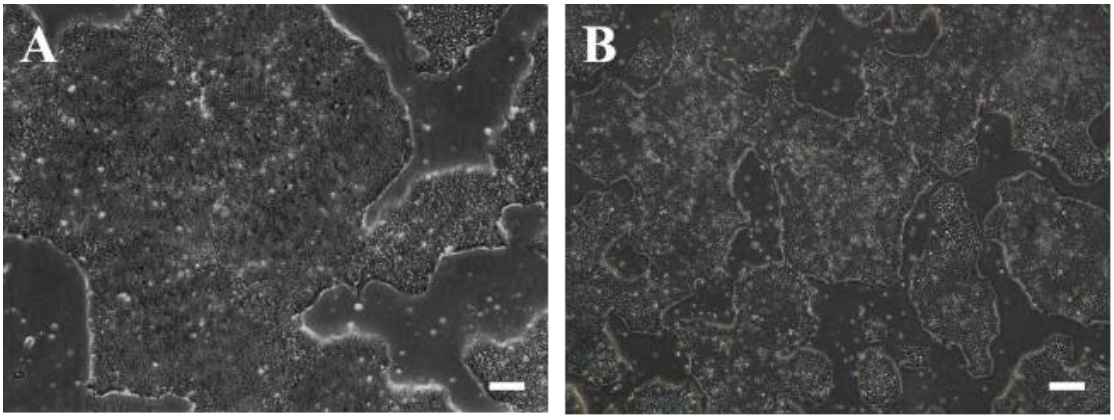

Figure 1: hiPSC colonies at approximately 85% confluency.
(A) Matrigel Plate; (B) Vitronectin Plate.
Scale bar: 200 µm.

2. Passage Ratio:
   Passage at a ratio of 1:5 to 1:20 based on cell growth status and experimental needs. If cells are normal, with uniform colony size and 85% confluency (Figure 1), a 1:10 passage ratio is recommended. If cell density is low, reduce the passage ratio; if density is high, increase the ratio.
3. Place the Matrigel-coated 6-well plate in the biosafety cabinet for about 1 hour to warm to room temperature (~25℃).
4. Prepare 2 mL/well of hPSC complete medium (NcEpic™ or NcTarget™) for the number of wells to be passaged. Add Blebbistatin (10 mM) at a 1:4000 ratio. Allow the medium to reach room temperature (~25℃).

TIPS: Add 0.5 µL of Blebbistatin (10 mM) to 2 mL of hPSC complete medium.

5. Remove the medium from the hiPSC wells. Add 2 mL/well of DPBS (without calcium and magnesium). Gently swirl and discard.
6. Add 2 mL/well of EDTA detachment solution to cover the well surface.
7. Incubate in a 37℃ incubator for 7-8 minutes.

   TIPS: (1) Observe cell changes under the microscope after 8 minutes. When most cells become bright and round, but have not yet detached or floated, stop the detachment (Figure 2C). If most cells are not yet bright, extend the detachment time (Figure 2A & 2B). (2) Ensure that the 6-well plate is in direct contact with the metal shelf of the incubator to maintain even heating. Do not stack the plates.

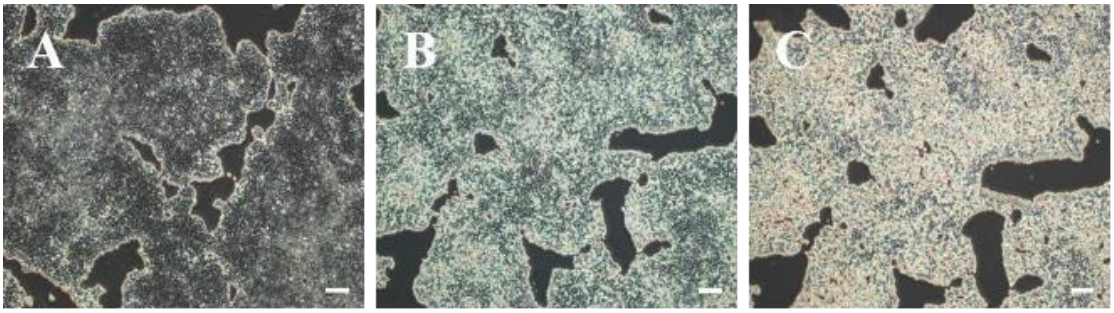

Figure 2: (A) Digestion for 4 min; (B) Digestion for 6 min; (C) Digestion for 8 min. Scale bar: 200 µm.

8. After dissociation, gently return the plate to the biosafety cabinet. Avoid shaking or disturbing the cells. Tilt the plate and discard the hPSC Dissociation Buffer.
9. Immediately add 2 mL/well of pre-warmed Blebbistatin + hPSC complete medium (NcEpic or NcTarget). Swirl the plate horizontally to detach the cells from the matrix.

   TIPS: (1) When adding Blebbistatin + hPSC complete medium (NcEpic™ or NcTarget™), gently pipette the cells 1-2 times. Do not exceed 2 pipetting actions to avoid breaking the cells into single cells.

   (2) Avoid scraping the cells. It is normal for some cells (10-15%) to remain attached. If a large number of cells remain attached, extend the detachment time.

   (3) Do not process more than one 6-well plate at a time. Once hPSC complete medium (NcEpic™ or NcTarget™) is added, quickly remove the plate. The EDTA detachment effect is rapidly terminated upon addition of hPSC complete medium, and cells will quickly reattach. hiPSCs should not remain in EDTA detachment solution for more than 15 minutes.
10. Seeding:
    10.1. Aspirate the Matrigel solution from the 6-well plate and add 2 mL/well of pre-warmed Blebbistatin+ **hPSC culture medium (NcEpic or NcTarget)**.
    10.2. Gently mix the cell suspension obtained in step 9 and evenly distribute it into the wells according to the predetermined passage ratio.

    TIPS: Alternatively, calculate the required number of cells for each passage per plate. Transfer the cells to a 15 mL centrifuge tube. Resuspend them in pre-warmed Blebbistatin+ hPSC culture medium (NcEpic™ or NcTarget™) to a final volume of 12 mL. Evenly distribute the suspension to a Vitronectin coated 6-well plate from which the coating solution has been aspirated.
11. Gently rock the 6-well plate horizontally in a cross pattern three times. Bring it to the incubator and rock it again in the cross pattern three more times. Culture overnight at 37°C with 5% CO2 concentration and saturated humidity.

12. Replace the medium with fresh hPSC complete medium (NcEpic or NcTarget) after 18-24 hours. Change the medium daily thereafter. Passage or harvest cells after 4-5 days. (Figures 3-4).

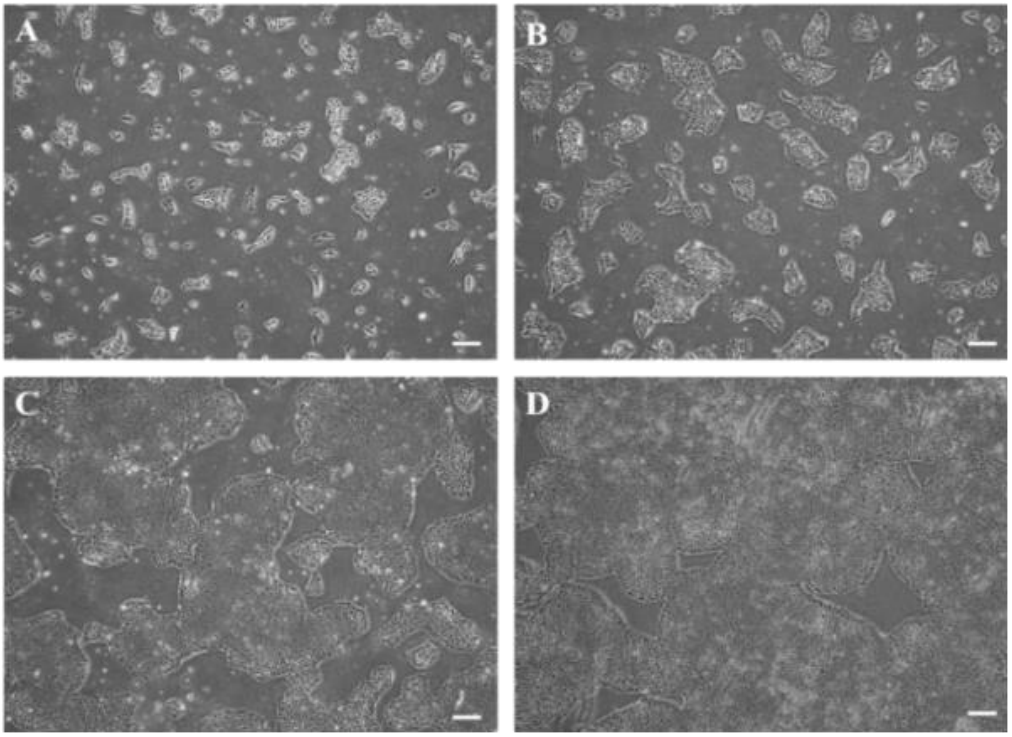

Figure 3: Morphology of hiPSCs cultured in NcEpic hPSC medium, Matrigel Plate. Scale bar: 200 μm.
A, B, C, D show hiPSC morphology on days 1, 2, 3, and 4 of culture, respectively.

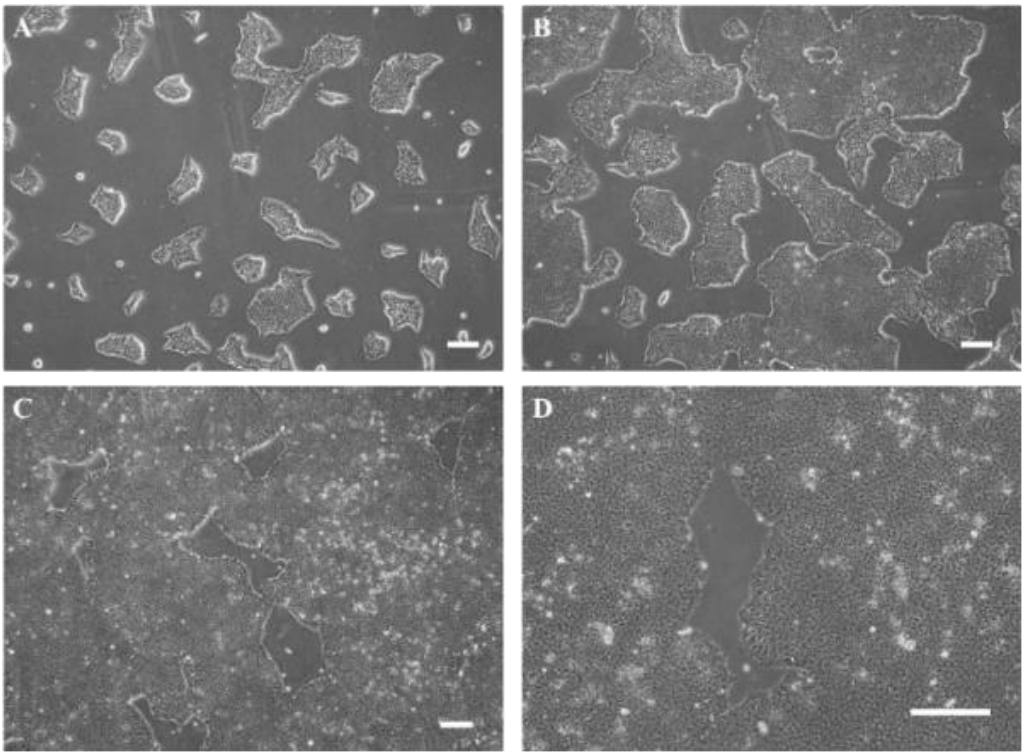

Figure 4: Morphology of hiPSCs cultured in NcTarget hPSC medium, Matrigel Plate. Scale bar: 200 μm.
A, B, C show hiPSC morphology on days 2, 3, and 4 of culture, respectively. D shows a magnified view of cells on day 4.

VII. Cryopreservation of hPSC

1. When the cell confluence reaches about 85% (Figures 1), cells can be harvest for cryopreservation. Typically, 2×106-4×106 viable cells per well of a 6-well plate can be collected and frozen in one cryovial.
2. Prepare the appropriate number of 1.5/2 mL cryovials and label properly.
3. Take out hPSC High-Efficiency Cryopreservation Medium from the 4°C refrigerator and equilibrate to room temperature. Mix thoroughly before use.

   TIPS: DMSO in the cryopreservation medium tends to settle at the bottom. Inadequate mixing may result in insufficient DMSO concentration initially and excessive concentration later, leading to instability in cryopreserved cells.
4. Aspirate the culture medium from the plate and add 2 mL/well of DPBS (without calcium and magnesium). Gently rock swirl several times and aspirate.
5. Add 2 mL/well of hPSC dissociation working solution. Place the plate in a 37°C incubator for 7-8 minutes (refer to Section VI, Step 7).
6. After digestion, gently take out the culture plate and aspirate the EDTA buffer.
7. Mix the pre-warmed hPSC cryopreservation medium thoroughly. Add 1 mL of cryopreservation medium to each well. Gently pipette to resuspend the cells, then rock the plate in a horizontal cross pattern three times. Transfer the cell suspension into the labeled 1.5/2 mL cryovials.
8. Place the vials in a cell freezing container and store at -80°C overnight. Transfer the vials to a liquid nitrogen tank for long-term storage the next day. Alternatively, use a programmable controlled rate freezer to cool the cells below -80°C before transferring to liquid nitrogen storage.

VIII. Problems and Solutions

• **Differentiation Observed in hiPSC Culture**
• Ensure hPSC culture medium (NcEpic™ or NcTarget™) is stored at 4°C and used within 2 weeks. Only pre-warm the amount of medium required for the current experiment to minimize temperature fluctuations and prevent degradation of ingredients in the medium. • If hPSC colonies exhibit good overall morphology with sporadic differentiated cells (<1%) at the edges, these cells can be removed during EDTA passaging. • Ensure that the size of the hPSC cell clusters during passaging is uniform, with the size of about 20 cells being ideal. For larger clusters, gently pipette no more than 3 times using a 5 mL pipette, applying light and even pressure to avoid cell damage or differentiation. • Avoid removing cells from the incubator for more than 15 minutes during observation. • If hPSC clones appear loose internally with irregular edges and differentiation exceeds 20%, discard the culture.
• **Can Dispase or Collagenase Be Used for hPSC Passaging?**
• Dispase or collagenase can be used, but digestion efficiency may be suboptimal, affecting post-passaging cell viability and potentially accumulating differentiated cells.. • For hPSCs cultured in the NcEpic™ or NcTarget™ system, non-enzymatic, gentle dissociation methods are recommended for passaging. • If single-cell dissociation is required for experiments, use Accutase for 5-10 minutes.
• **hPSCs Fail to Adhere or Exhibit Low Adhesion After Passaging**

• Avoid excessive passaging ratios (>1:20). • EDTA dissociation time should not be too long. While some cell lines may require extended dissociation beyond 8 minutes, do not exceed 15 minutes. • Avoid excessive pipetting (>3 times) to prevent clusters disruption or cell damage. • Ensure culture plates are coated with Vitronectin, Matrigel, or other matrices suitable for pluripotent stem cell growth. • ROCKi is necessary in the medium after passaging.
• **Cells detach after medium change**
• Perform the first medium change 18-24 hours after seeding to ensure proper cell adhesion. • Handle medium changes gently to avoid dislodging cell clusters from the matrix. • If cell seeding density is very low (e.g., for cell cloning experiments), avoid medium changes for 2-3 days, ensuring the medium contains ROCKi.
• **Uneven distribution of hiPSC clusters in wells**
• Ensure the coating matrix is evenly distributed across the bottom of the culture vessel. • During passaging, ensure cells are evenly dispersed. After rocking the plate in a horizontal cross pattern, avoid moving the plate to prevent cell aggregation in the center. • After placing the plate in the incubator, rock it again in a horizontal cross pattern to ensure even distribution.