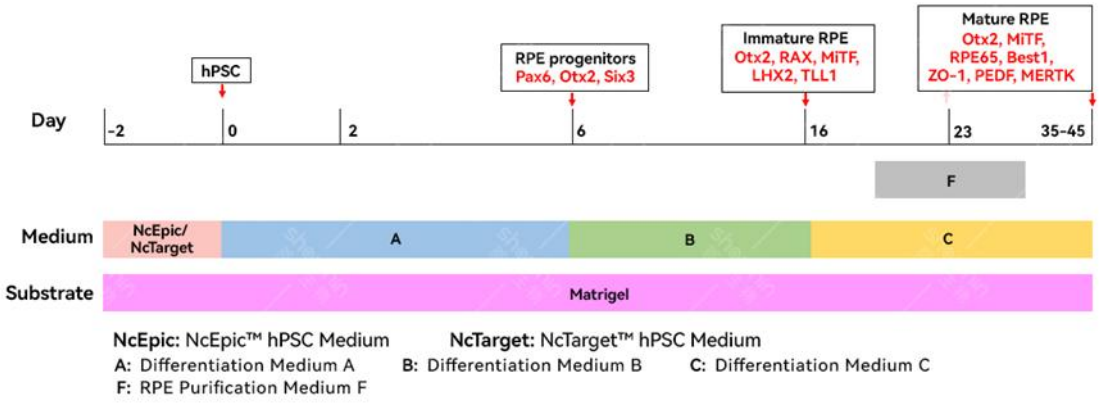
hPSC-RPE Differentiation Kit

Product Manual

Catalog#RP01016 1 Kit

Product Introduction

The hPSC-RPE Differentiation Kit is designed for differentiating human pluripotent stem cells (hPSCs) into retinal pigment epithelial cells (RPE). The kit includes differentiation medium, RPE purification buffer, and cryopreservation medium. Using this kit, high-purity RPE cells (>95% $MITF^+/ZO\text{-}1^+$) can be obtained from hPSCs. hPSC-RPE cells are suitable for scientific research, drug screening, and the establishment of disease-related experimental models.



Product Information

Table 1. hPSC-RPE Differentiation Kit Product System

Product	Cat.No.	Amount	Storage
hPSC-RPE Differentiation Kit*	RP01016	1 Kit	Basal medium: 2–8 °C Supplements: -80 °C or -20 °C
hPSC-RPE Progenitor Maturation Medium*	RP01016-H	1 Kit	

*** Each kit ultimately yields approximately 1×10^7 mature RPE cells.**

*** The complete differentiation medium is prepared by mixing the basal medium and additives, and can be stored at 2–8 °C and used up within 2 weeks.**

Recommended 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 Digestion Solution	Shownin	RP01021
0.25% Trypsin Solution	Shownin	RP02011
Trypsin Inhibitor	Shownin	RP02012
CORNING® MATRIGEL® Matrix	Corning	354277
DMEM/F12 Medium	Thermo Sci.	11330
DPBS, no calcium, no magnesium	Thermo Sci.	14190144
6/12/14-Well Plate	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
10 µL / 200 µL / 1000 µL Pipette Tips	Rainin.	N/A
Freezing Container	Thermo Sci.	5100-0001

hPSC-RPE Differentiation

2.1 Reagent Preparation

Table 3. hPSC-RPE Differentiation Kit Product Information

Product Information	Cat. No.	Amount	Storage
hPSC-RPE Differentiation Kit* contains:	**RP01016**	**1 Kit**	
RPE Differentiation Supplement A (50×)	RP01016-A	800 µL	-80 °C or -20 °C
RPE Differentiation Supplement B (50×)	RP01016-B	1.2 mL	
RPE Differentiation Supplement C (50×)	RP01016-C	2 mL	
RPE Differentiation Basal Medium D	RP01016-D	100 mL	2–8 °C
RPE Differentiation Basal Medium E	RP01016-E	100 mL	
RPE Purification Buffer F	RP01016-F	10 mL	
RPE cryopreservation medium G	RP01016-G	10 mL	

***Each kit ultimately yields approximately 1 × 10^7 mature RPE cells.**

***Each kit is sufficient for 6 wells of a 12-well plate or 3 wells of a 6-well plate.**

***After mixing basal medium and supplements, the prepared differentiation complete medium can be stored at 2–8 °C for up to 2 weeks.**

2.1.1 Thaw RPE Differentiation Supplements A, B, and C at 4 °C. **Do not thaw at 37 °C.**

2.1.2 Prepare **Differentiation Complete Medim A, B, and C (1×)** in a biosafety cabinet according to Table 4.

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

Tips: Aliquot RPE Differentiation Supplements A, B, and C for freezing if needed. Avoid more than 2 freeze-thaw cycles.

Table 4. Reagent preparation instructions for the hPSC-RPE Differentiation Kit

Type	Components	Final Concentration
Differentiation Complete Medium A/B (1×)	RPE Differentiation Supplement A (50×)/B (50×)	1×
	RPE Differentiation Basal Medium D	
Differentiation Complete Medium C (1×)	RPE Differentiation Supplement C (50×)	1×
	RPE Differentiation Basal Medium E	

2.2 hPSC-RPE 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 -2**, for a 12-well plate, when hPSCs reach 85% confluency, passage and seed cells into new wells at a density of 1×10^5 cells/well. Maintain culture for 2 Days, replacing the medium daily.

Tips: The protocol applies to other vessels with a seeding density of 2×10^4 cells/cm². Initiate differentiation after 5 passages with good cell status.

2.2.3 **Day 0**, aspirate the medium and add 1 mL/well of **Differentiation Complete Medium A**. Replace the medium daily until Day 6 (Day 0–5).

2.2.4 **Day 6**, aspirate **Medium A** and add 1 mL/well of **Differentiation Complete Medium B**. Replace the medium daily until Day 16 (Day 6–15).

2.2.5 **Day 16**, aspirate **Medium B** and add 1 mL/well of **Differentiation Complete Medium C**. Replace the medium daily until Day 23 (Day 16–23).

2.2.6 **Day 23**, aspirate **Medium C** and add 1 mL/well of DPBS (without calcium and magnesium) for one wash. Then, add 1 mL of **RPE Purification Buffer F** and incubate in a 37 °C, 5% CO_2, saturated humidity incubator for 6–8 minutes to allow the heterogeneous cells to completely detach from the bottom of the culture dish.

Tips: The heterogeneous cells are fibroblast-like and are relatively easy to separate from the RPE cells. During digestion, the culture dish can be taken out of the incubator and observed under a microscope to check the condition of the heterogeneous cells. The dish can be gently shaken by hand to accelerate the separation of the heterogeneous cells.

2.2.7 After all the heterogeneous cells have floated up, aspirate the supernatant. Then, add 1 mL/well of DPBS (without calcium and magnesium) and wash three times to ensure complete removal of the heterogeneous cells.

Tips: This step is effective in removing nearly all the heterogeneous cells.

2.2.8 Add 2 mL/well of **Differentiation Complete Medium C** and maintain the culture until Day 35–45, with medium changes every 3–4 Days. The RPE cells harvested during this period (Day 35–45) are suitable for a range of scientific research applications.

2.2.9 **Cryopreservation:** According to experimental needs, the obtained hPSC-RPE cells can be cryopreserved. Aspirate the supernatant, add 1 mL/well of DPBS (without calcium and magnesium) for one wash, then add 1 mL of **0.25% Trypsin Solution** and incubate in a 37 °C, 5% CO_2, saturated humidity incubator for 3–5 minutes. When most of the RPE cells turn brighter, remove **the trypsin solution**, add 1 mL of **trypsin inhibitor** to each well, and gently pipette 3–5 times. Collect the RPE cells into a 15 mL centrifuge tube and centrifuge at **180 × g** for **5** minutes.

Tips: During cell dissociation, it is important to monitor the cell condition. Terminate dissociation when most of the cells turn brighter; at this point, most cells will not have detached. After removing the trypsin solution, add the trypsin inhibitor and gently pipette 3–5 times to collect the cells. If most of the cells have detached, directly add the trypsin inhibitor and gently pipette 3–5 times to collect the cells.

2.2.10 Aspirate the supernatant, add an appropriate amount of **RPE cryopreservation medium G** to resuspend the RPE cells and count them. Then, cryopreserve the cells at a certain density (e.g., 3×10^6 cells/tube).

hPSC-RPE Cell Thawing and Maturation culture

3.1 Reagent Preparation

Table 5. hPSC-RPE Cell Maturation Medium Product System

Product Information	Cat.No.	Norm	Storage
hPSC-RPE Cell Maturation Medium Contains:	**RP01016-H**	**1 Kit**	
RPE Differentiation Supplement C (50×)	RP01016-C	2 mL	-80 °C or -20 °C
RPE Differentiation Basal Medium E	RP01016-E	100 mL	2–8 °C

3.1.1 Thaw RPE Differentiation Supplement C at 4 °C. **Do not thaw at 37 °C.**

3.1.2 In a biosafety cabinet, prepare **the RPE Cell Maturation Complete Medium (1×)** according to Table 4.

RPE Differentiation Basal Medium E: 98 mL

RPE Differentiation Supplement C (50×): 2 mL

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

Tips: RPE Differentiation Supplement C can be aliquoted and stored frozen if needed. Avoid more than 2 freeze-thaw cycles.

3.2 Thawing and Maturation culture of hPSC-RPE Cells

3.2.1 Preheat a water bath to 37 °C. Equilibrate Matrigel-coated 6-well plates in a biosafety cabinet for 30 minutes (15–30 °C).

3.2.2 Add 6 µL of **Blebbistatin** (10 mM) to 6 mL of **RPE Maturation Complete Medium** (1:1000 ratio) and equilibrate to room temperature (15–30 °C).

3.2.3 Retrieve 1 tube of frozen **hPSC-RPE cells** from the liquid nitrogen tank and immediately place it in the 37 °C water bath, gently shaking the vial by hand. Thaw the cells within 1 minute, and remove the vial when the ice crystals in the cell suspension are nearly completely gone.

3.2.4 Wipe the surface of the cryovial with 75% alcohol and transfer it into the biosafety cabinet. Transfer the cell suspension into a pre-prepared 15 mL centrifuge tube. Using a pipette, slowly add 10 mL of DMEM/F12 dropwise to the thawed cell suspension while gently mixing the cells. Centrifuge at 180 × g for 5 minutes.

3.2.5 Aspirate the supernatant, gently resuspend the pellet in 6 mL of **Blebbistatin + RPE Maturation Complete Medium**, and avoid excessive pipetting.

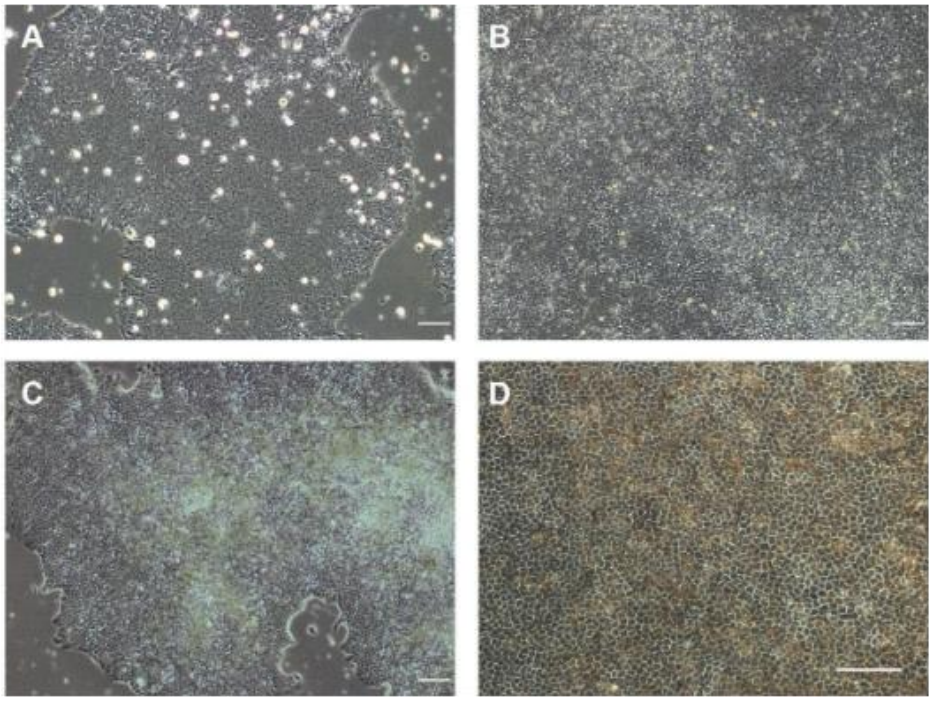
Tips: The recommended seeding density for RPE cells is 1–2 × 10^5 cells/cm^2. Ensure thorough mixing when seeding.

3.2.6 Aspirate Matrigel from 2 wells of the 6-well plate and seed cells at 3 mL/well dropwise.

3.2.7 Gently swirl the plate in a cross pattern to ensure even distribution.

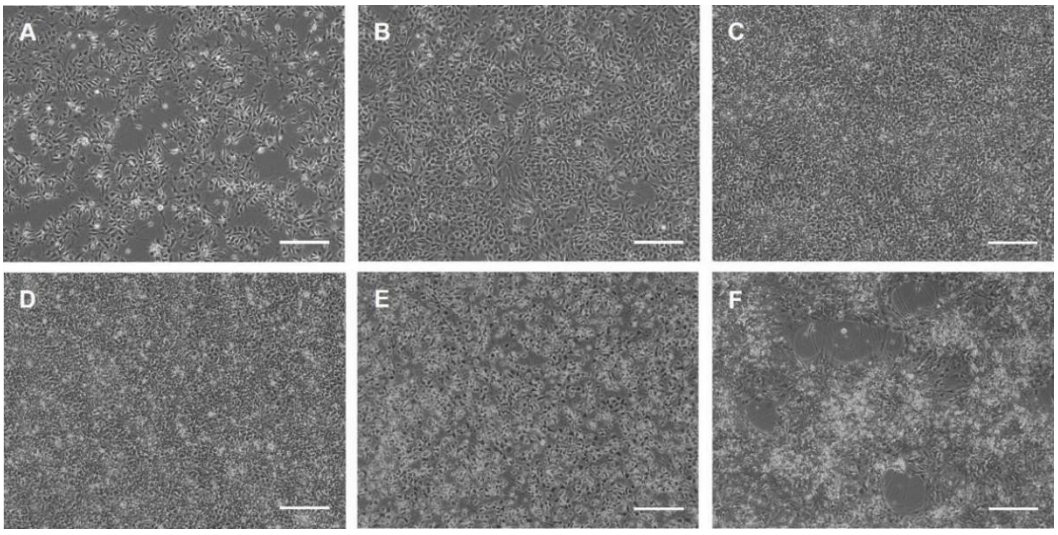
3.2.8 Place the plate in a 37 °C, 5% CO_2, saturated humidity incubator, and shake gently in a horizontal "cross" direction three times. Maintain culture.

3.2.9 Replace the **RPE Maturation Complete Medium** after 18–24 hours, then every 2 Days (3 mL/well). Typical RPE morphology appears by Day 8, and melanin secretion becomes evident by Day 10.



Morphology of hPSC-RPE Differentiation Process (Scale bar: 200 μm)

A: Day 0 (hPSC); B: Day 6 (RPE Progenitors); C: Day 16 (Immature RPE); D: Day 45 (Mature RPE).



Morphology of hPSC-dopaminergic neural progenitor cells during revival, maintenance culture, and maturation. Scale bar: 200 μm.

Figure 2A, B, C: Morphology of hPSC-dopaminergic neural progenitor cells (RPE) on Day 1, 2, and 4, respectively.

Figure 2D, E, F: Morphology of RPE cells differentiating into mature dopaminergic neurons (mDAN) on Day 1, 9, and 30, respectively.