Human iPSC-Derived Liver Organoid Differentiation Kit

Human iPSC-Derived Liver Organoid Differentiation Kit
Cat. No. : RIPO-RWM009K

Product Description

Human iPSC-Derived Liver Organoid Differentiation Kit (Cat. No. RIPO-RWM009K) allows hESC or hiPSC to differentiate into liver organoids. Liver organoids are three-dimensional *in vitro* models with a cellular composition and structural organization that are representative to the human liver. This kit can produce 24 liver organoids that show expression of hepatocytes (HFN4a), hepatobiliary tracts (CK19), albumin (ALB) and vascular endothelial cell (CD31).

Product Specification

The basic medium of this differentiation kit is a serum-free, well-defined medium with minimal batch variation to which differentiation factors are added. This medium does not contain antibiotics, the addition of which may affect organoid differentiation.

Product Information

Name	Component #	Size	Storage	Shelf Life
Liver Basal medium A	RIPO-RWM009K-C01	9 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Supplement A	RIPO-RWM009K-1-C01	1 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Basal Medium B	RIPO-RWM009K-C02	9 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Supplement B	RIPO-RWM009K-1-C02	1 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Basal Medium C	RIPO-RWM009K-C03	13.5 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Supplement C-1	RIPO-RWM009K-1-C03	1.2 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Supplement C-2	RIPO-RWM009K-1-C04	0.3 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Basal Medium D	RIPO-RWM009K-C04	22.5 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Supplement D-1	RIPO-RWM009K-1-C05	2 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Liver Supplement D-2	RIPO-RWM009K-1-C06	0.5 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label

Materials Required but Not Included

- mTeSR Plus (STEMCELL Technologies, # 100-0276)
- TrypLE™ Express Enzyme (1X), phenol red (Gibco, #12605-028)
- DMEM/F12 (Gibco, #11320-033)
- D-PBS (Without Ca++ and Mg++)

- Ultra-Low Adherent 96-well Plate
- Ultra-Low Adherent 6-well Plate
- TC-treated 6-well plate
- Hemocytometer
- Trypan Blue Solution

Equipment Required

- Incubator (37°C, 5% CO_2)
- Low-speed Centrifuge (with a swinging bucket rotor and an adaptor for plate holders)
- Incubated Orbital Shaker (any brand, 2 cm shaking diameter)
- Biosafety Cabinet

Protocol Diagram

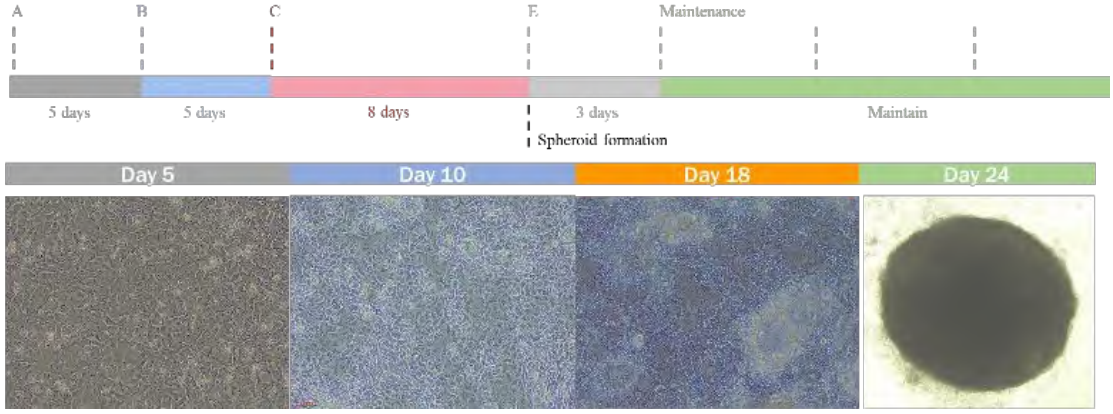


Figure 1. Liver Organoid Differentiation Process

The color differs for each component of the differentiation kit. The dashed line represents the time of medium changes. Morphology of liver organoid at each stage of differentiation could be observed.

Media Preparation

Use sterile technique when performing the following manipulations:

Medium	Component	Volume	IN-USE STORAGE/STABILITY
Medium A (10 ml)	Basal Medium A	9 ml	Mix completely the Basal Medium A and Supplement A to get Medium A. Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement A	1 ml	
Medium B (10 ml)	Basal Medium B	9 ml	Mix completely the Basal Medium B and Supplement B to get Medium B. Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement B	1 ml	
Medium C (15 ml)	Basal Medium C	13.5 ml	Mix completely the Basal Medium C, Supplement C-1, and Supplement C-2 to get Medium C. Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement C-1	1.2 ml	
	Supplement C-2	0.3 ml	
Medium D (25 ml)	Basal Medium D	22.5 ml	Mix completely the Basal Medium D, Supplement D-1, and Supplement D-2 to get Medium D. Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement D-1	2 ml	
	Supplement D-2	0.5 ml	

Note: Please do not heat the complete medium (mixture of basal medium and supplement). Use it directly as cold as 2-8°C.

Directions for Use

Please read the entire protocol before proceeding.
Use sterile technique when performing the following protocols.

Note: Before liver organoid culturing, please make sure that the culture system you use is in 6-well plate coated by Matrigel mTeSR-based, and the cell confluence should exceed 50%. If your culture system is not mTeSR, please make sure that you have transferred your cells to the mTeSR system for at least 4 passages.

Liver Organoid Differentiation

Induction

1. Aspirate medium from iPSC culture and add 3 ml of medium A to each well, incubate at 37°C, 5% CO_2 for 5 days. Change the medium A every other day.
   Note: Warm medium A at room temperature before use.
2. Remove medium A and add 3 ml of medium B in each well, incubate at 37°C, 5% CO_2 for 5 days. Change the medium B every other day.
   Note: Warm medium B at room temperature before use.
3. Remove medium B and add 3 ml of medium C in each well, incubate at 37°C, 5% CO_2 for 8 days. Change the medium C every other day.
   Note: Warm medium C at room temperature before use.
4. Aspirate medium C and wash the well with 3 ml of D-PBS (without Ca++ and Mg++) 3 times, 1 min each time.
   Note: Warm D-PBS (without Ca++ and Mg++) at room temperature before use.
5. Aspirate D-PBS and add 3 ml of TrypLE in each well.
6. Incubate about 10-15 mins for digestion of iPSCs to single cells. Use pipettes to pipet cells for

obtaining single cells.

Note: Incubation time may vary when using different cell lines or different cell dissociations.

7. Transfer the cell suspension to a sterile centrifuge tube and add DMEM/F12 (twice the volume of the digestion solution) to terminate the digestion reaction. Centrifuge at 300 g, 4 °C for 3 mins.

Box1: If you want to cryopreserve your liver organoids

After step 7, please using the following freeze and thaw protocol:

Freezing

1. Gently suspend the cells with 1 ml of CELLBANKER® cryopreservation medium.
2. Dispense the cell suspension in 1 ml aliquots to cryogenic vials that have been labeled with the cell line name, cell concentration, passage date and other essential information.
3. Place the vials directly in -80 ℃ for storage. If necessary, transfer the frozen vials to a liquid nitrogen storage tank after the vials have been frozen for at least 24 hours.

Thawing

1. Remove the frozen cell from storage and quickly thaw in a 37 ℃ shaking water bath.
2. Immediately dilute and gently mix each 1 ml of cells with 10 ml of complete cell culture medium.
3. Gently pellet the cells centrifugation (3-5 mins at 1,000 - 2,000 rpm, 4 ℃). Remove the supernatant aspirator.
4. Continue to Step 8.

Sphere Formation

8. Remove the supernatant and add 2-3 ml medium D to resuspend cells.
   Note: Warm medium D at room temperature before use.
9. Count cells using Trypan Blue and a hemocytometer.
10. Add an appropriate volume of medium D to acquire a final concentration of $5x10^5$ cells/ml
11. Add 200 µl of cell suspension into each well of a 96-well round-bottom ultra-low adherent plate ($1x10^5$ cells/well). Incubate the plate at 37°C, 5% CO_2 for 24 h.
12. Observe under the microscope at 24 h of incubation. If spheres are formed, then incubate for another 48 h; if spheres are not formed, centrifuge the plate at 300 g, 4 °C for 3 mins and then incubate for another 48 h.

Liver Organoid Expansion and Maintenance

13. Transfer all liver organoids into ultra-low adherent 6-well plate (the maximum number is 24 organoids per well) and add 5 ml Human iPSC-Derived Liver Organoid Expansion and Maintenance Medium (Cat. No. RIPO-RWM010) per well. Then put the plate in incubated orbital shaker at 37°C, 5% CO_2 with the speed of 100 rpm.
14. Change the Human iPSC-Derived Liver Organoid Expansion and Maintenance Medium (Cat. No. RIPO-RWM010) fully every three days with a volume of 5 ml.

Related Products

Product	Cat. No.
Human iPSC-Derived Liver Organoid Expansion and Maintenance Medium	**RIPO-RWM010**

Product Validation

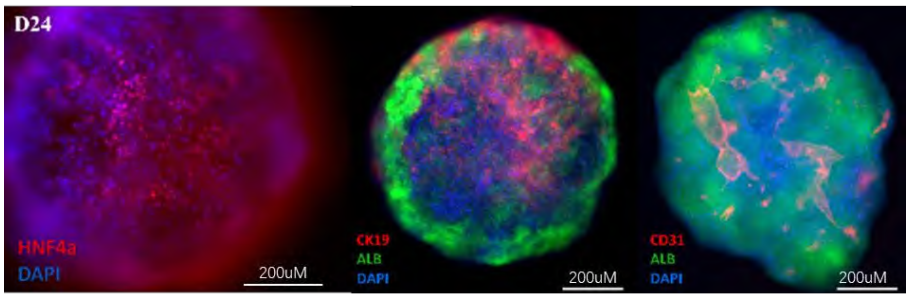


Fig. 2. Immunofluorescence Staining of Liver Organoids

The liver organoids show expression of hepatocytes (HFN4a), hepatobiliary tracts (CK19), albumin (ALB) and vascular endothelial cell (CD31).

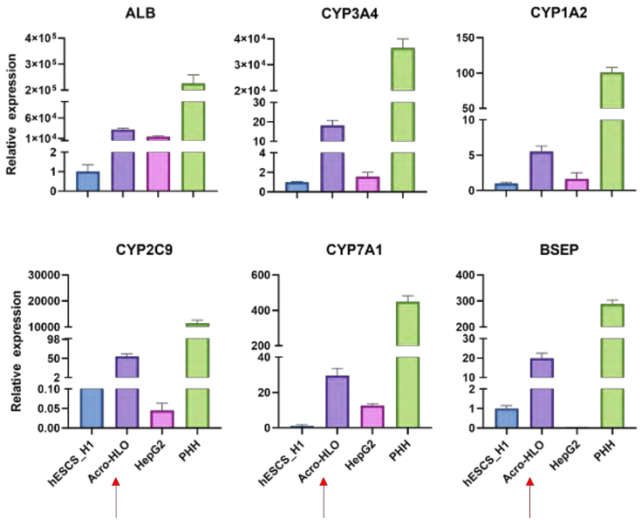


Fig. 3. Functional Assessment of Liver Organoids

Liver organoids show better performance than HepG2 cell line in terms of the expression of albumin, CYP3A4, CYP1A2, CYP2C9, CYP7A1 and BSEP.

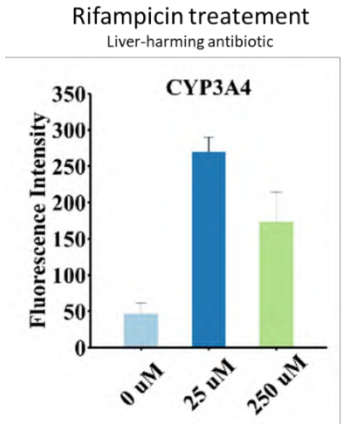


Fig. 4. Functional Assessment of Liver Organoids

Liver organoids show responses to Rifampicin treatment as indicated by the increased fluorescence intensity of CYP3A4.