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 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 is representative to the human liver. This kit can produce 48 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	10 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	10 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	22.5 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	12.5 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)
- Gentle Cell Dissociation Reagent (STEMCELL Technologies, #100-0485)
- DMEM/F12 medium (Gibco, #11320-033)
- D-PBS (Without Ca++ and Mg++)

- Ultra-Low Attachment 96 Well Plate
- Ultra-Low Attachment 6 Well Plate
- Orbital shaker (any brand, 2 cm shaking dimeter)
- Hemocytometer
- Trypan blue

Equipment Required

- Incubator (37°C, 5% CO_2)
- Low-speed centrifuge with a swinging bucket rotor with an adaptor for plate holders
- Incubated shaker
- Biosafety cabinet

Protocol Diagram

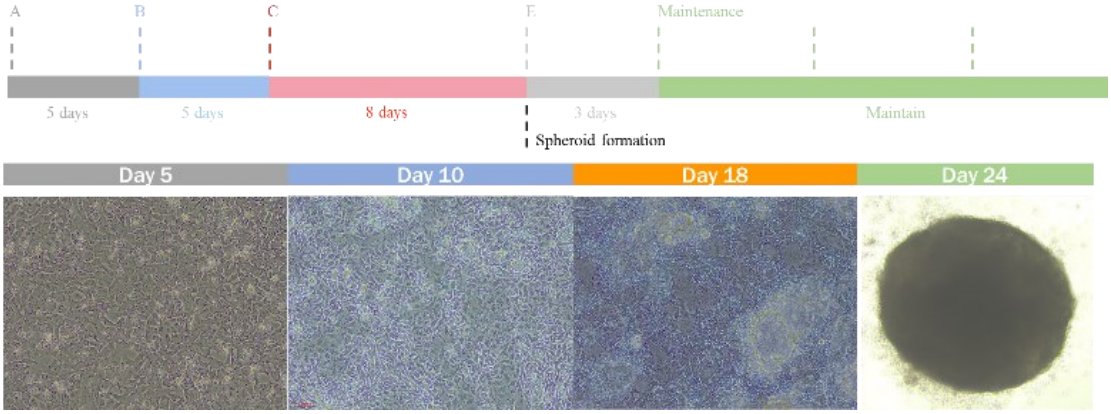

Figure 1. liver Organoid Differentiation Process

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

Preparation of Media

Use sterile technique when performing the following manipulation

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 B. 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 (24 ml)	Basal Medium C	22.5 ml	Mix completely the Basal Medium C and Supplement C to get Medium B. 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 (15 ml)	Basal Medium D	12.5 ml	Mix completely the Basal Medium D and Supplement D to get Medium C. 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 beyond 90%. 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

1. Aspirate medium from hPSC culture and add 3 ml of medium A at each well and incubate at 37°C, 5% CO_2 for 5 days. Change the medium A every other day.
2. After 5 days, remove the 3 ml medium in each, add 3 ml of medium B in each well and incubate at 37°C, 5% CO_2 for 5 days. Change the medium B every other day.
3. After 5 days, remove medium from hPSC culture and add 3 ml of medium C in each well and incubate at 37°C, 5% CO_2 for 8 days. Change the medium C every other day.
4. After 8 days, aspirate medium from hPSC culture and wash the well with 3 ml of pre-warmed D-PBS (Without Ca++ and Mg++) 3 times, 1 min each time.
5. Aspirate PBS and add 2 ml of Gentle Cell Dissociation Reagent for each well.
6. Incubate about 10-15 minutes for digestion of iPSCs to single cells.

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

7. Add double volume DMEM/F12 medium of dissociation reagent and use pipettes to pipet cells for obtaining single cells and centrifuge at 300 g, 4 °C for 3 minutes
8. Remove the supernatant and add 2-3 ml medium D to resuspend cells.
9. Count cells using Trypan Blue and a hemocytometer.
10. Add appropriate volume of medium D to acquire final concentration of 50000 cells/ml
11. Add 200 µl of cell suspension into each well of a 96-well round-bottom ultra-low attachment plate (10000 cells/well). Incubate the plate at 37°C, 5% CO2 for 24 h.
12. Centrifuge the ultra-low attachment plate at 300 g, for 3 minutes if the formation of sphere is not observed after 24 h. procced the incubation for another 48 h.
13. After the last day of incubation with medium D, transfer all liver organoids into 125 ml flask (the maximum number is 96 organoids per flask) and add 30 ml medium M-M per flask. Then put the flask in Incubated shaker at 37°C, 5% CO2 with the speed of 100 rpm.
14. Change the medium M-M fully every three days with the volume of 30 ml.

Related Products

Product	Cat. No.
Liver Organoid maintenance medium	**RIPO-RWM010**

Product Validation

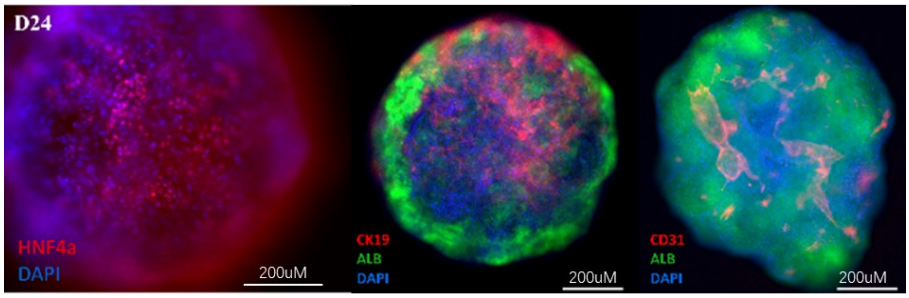

Figure 2. Immunofluorescence staining of liver organoids.

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

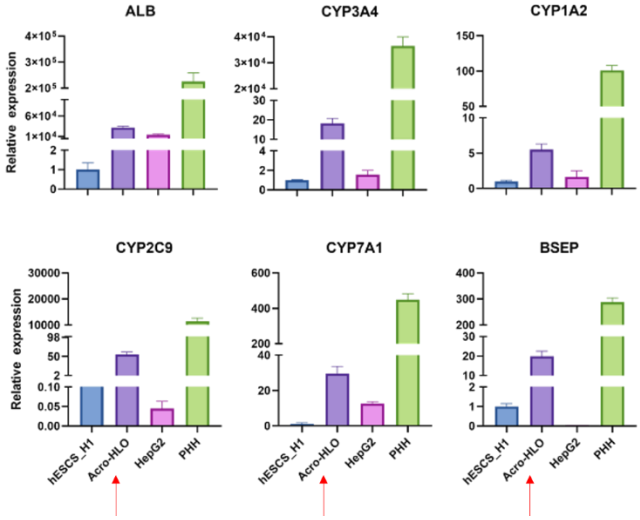

Figure 3. Functional assessment of liver organoids.

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

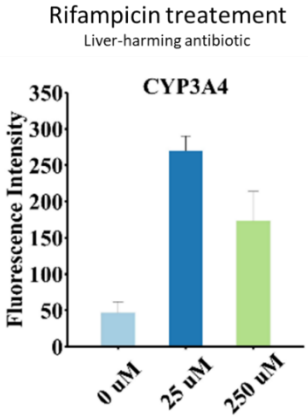

Figure 4. Functional assessment of liver organoids.

Liver organoid show response to Rifampicin treatment as indication by the increase fluorescence intensity of CYP3A4.