Human iPSC-Derived Intestinal Organoid Differentiation Kit

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Cat. No. : RIPO-IWM005K

Product Description

Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) allows hESC or hiPSC to differentiate into intestinal organoids. Intestinal organoids are three-dimensional *in vitro* models with a cellular composition and structural organization that is representative to the human intestine regions. Organoids generated using Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) feature various types of cells, including intestinal epithelial cells, mesenchymal cells, enterocytes, Paneth cells, goblet cells, etc. These intestinal organoids show intestine crypt like structure, villi and microvilli like structure, as well as normal intestinal function validated by the absorption of fatty acid and glucose.

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
Medium A	RIPO-IWM005K-1-C01	10 ml	-20 °C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium B	RIPO-IWM005K-C01	13 ml	4 °C	Stable for 1 year from date of manufacture (MFG) on label
Supplement B	RIPO-IWM005K-1-C02	2 ml	-20 °C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium C	RIPO-IWM005K-C02	13 ml	4 °C	Stable for 1 year from date of manufacture (MFG) on label
Supplement C	RIPO-IWM005K-1-C03	2 ml	-20 °C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium D	RIPO-IWM005K-C03	100 ml	4 °C	Stable for 1 year from date of manufacture (MFG) on label
Supplement D	RIPO-IWM005K-1-C04	10 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
- D-PBS (Without Ca++ and Mg++)
- Ultra-Low Adherent 96-well Plate

- Ultra-Low Adherent 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 dimeter)
- Biosafety Cabinet

Protocol Diagram

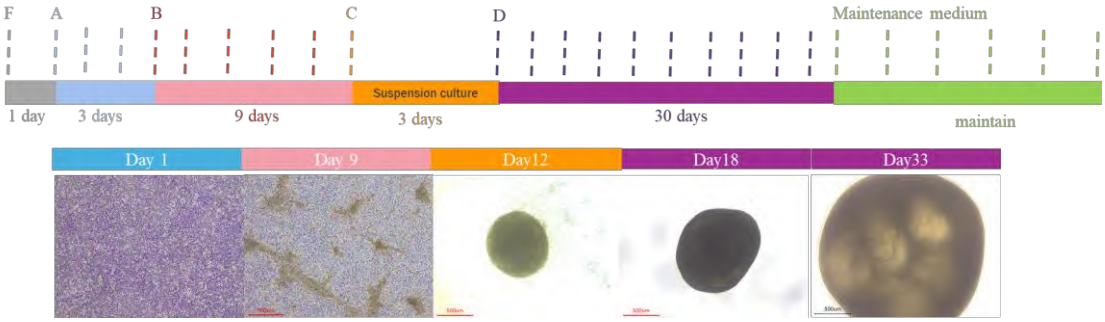

Fig. 1. Intestinal Organoid Differentiation Process

The color differs for each component of the differentiation kit. The dashed line represents the time of medium changes. Morphology of intestinal 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 B (15 ml)	Basal Medium B	13 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	2 ml	
Medium C (15 ml)	Basal Medium C	13 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	2 ml	
Medium D (110 ml)	Basal Medium D	100 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	10 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 intestinal 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 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.

Intestinal Organoid Differentiation

Induction

1. Aspirate medium from iPSC culture and add 3 ml of medium A at each well and incubate at 37 °C, 5% CO_2 for 72 h.
2. After 72 h, change the medium by 3 ml of medium B in each well and incubate at 37 °C, 5% CO_2 for 9 days. Change the medium B every other day. Collect all the medium B that are removed.
3. After 9 days, aspirate and **collect** the last medium B from iPSC culture. Wash the well with 3 ml of pre-warmed D-PBS (Without Ca++ and Mg++) 3 times, 1 min each time.
   Note: Collect all the medium B in the centrifuge tube.
4. Centrifuge the collected medium B at 300 g, 4 °C, removed the supernatant and add 12 ml of TrypLE to the tube to resuspend the cell clusters.
5. Aspirate D-PBS from the 6 well plate and add 2 ml of resuspended cell reagent in 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.

Box1: If you want to cryopreserve your intestinal 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 minutes 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 C to resuspend cells.
9. Count cells using Trypan Blue and a hemocytometer.
10. Add appropriate volume of medium C to acquire final concentration of 5 x 10^5 cells/ml.
11. Add 200 µl of cell suspension into each well of a 96-well round-bottom ultra-low adherent plate (1 x 10^5 cells/well), incubate 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 for 3 minutes and then incubate for another 48 h.
13. After the last day of incubation with medium C, transfer all intestinal organoids into ultra-low adherent 6-well plate (the maximum number is 24 organoids per well) and add 5 ml medium D per well. Then put the well on the orbital shaker with the speed of 100 rpm and incubate at 37 °C, 5% CO_2 for 72 h.
14. Change the medium by 5 ml medium D each well and incubate at 37 °C, 5% CO_2.
    Repeat medium D change every 3 days for another 8 times (change medium D 10 times in total).

Intestinal Organoid Maturation and Maintenance

15. After 45 days of differentiation, remove medium D, add 5 ml of medium MM and incubate at 37 °C, 5% CO_2.
16. Full medium change of 5 ml medium MM every 3 days.

Related Products

Product	Cat. No.
Human iPSC-Derived Intestinal Organoid Maintenance Kit	RIPO-IWM006

Validation Data of Intestinal Organoids

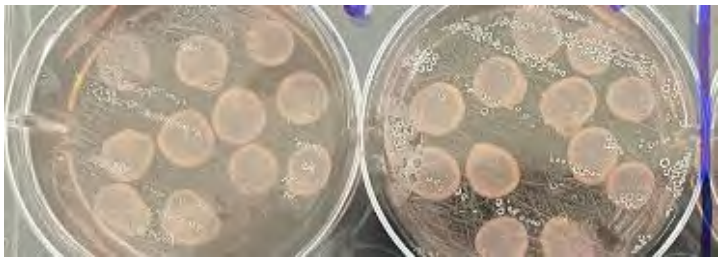

Fig. 2. Organoid Morphology

The intestinal organoids differentiated using the Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) show regular peristalsis.

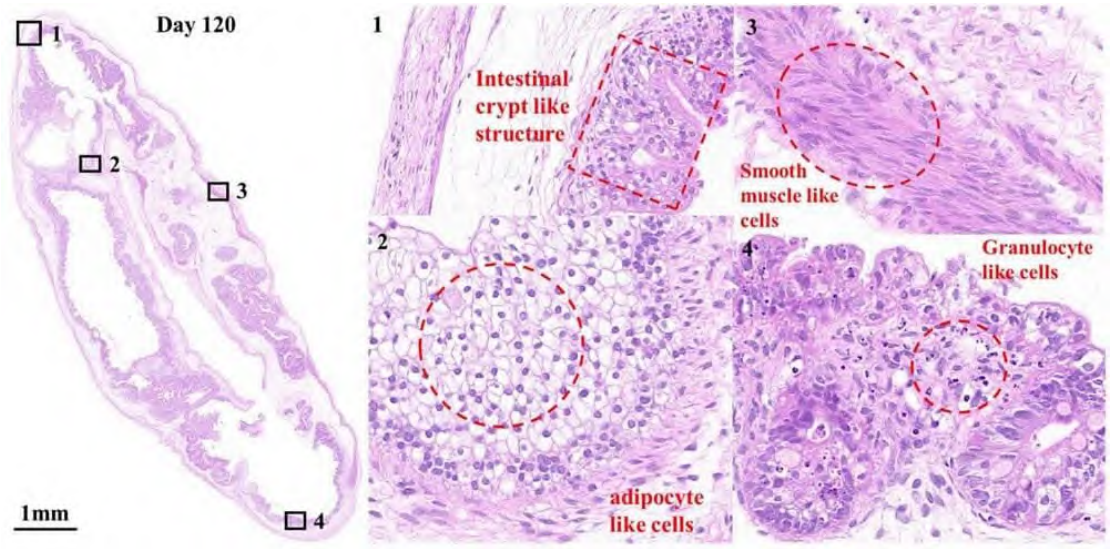

Fig. 3. Organoid Histology

Observation of granulocyte-like cells, adipocyte like cells, smooth muscle like cells and intestinal crypt like structure by morphology on day 120 intestinal organoids.

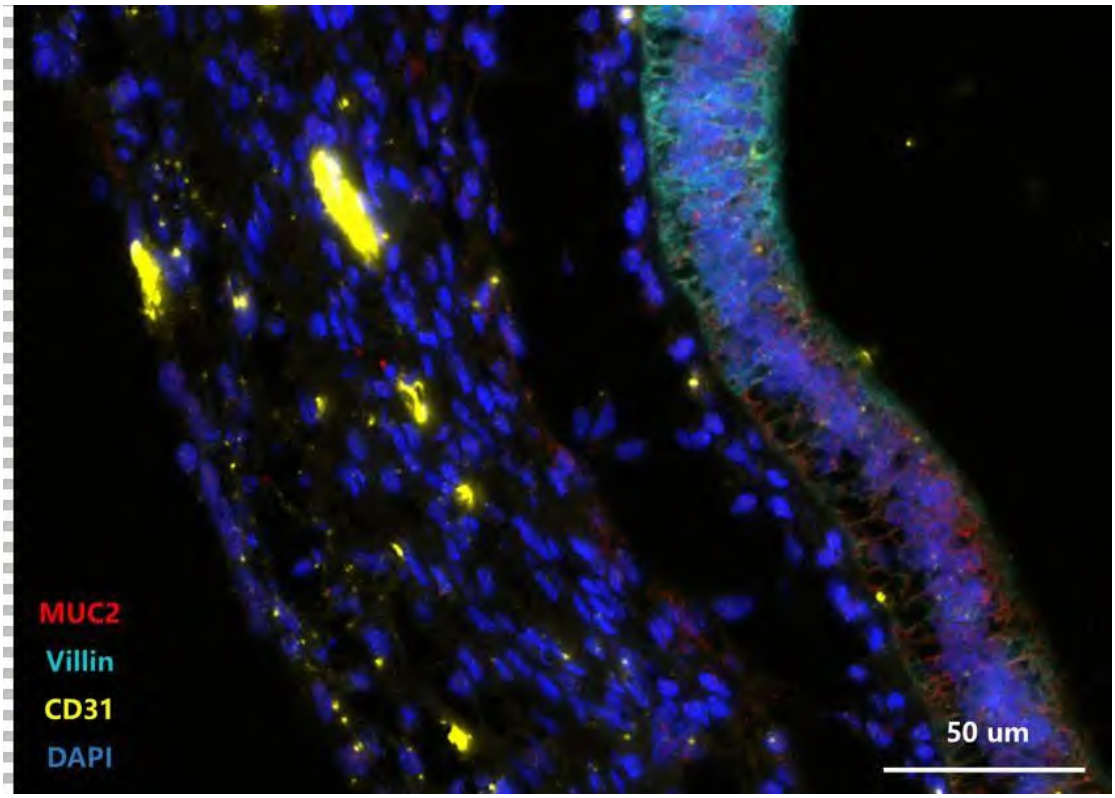

Fig. 4. Marker Expression

The intestinal organoids differentiated using the Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) show expression of goblet cells (mucus-producing, MUC2), brush borders (Villin) and endothelial cells (CD31).

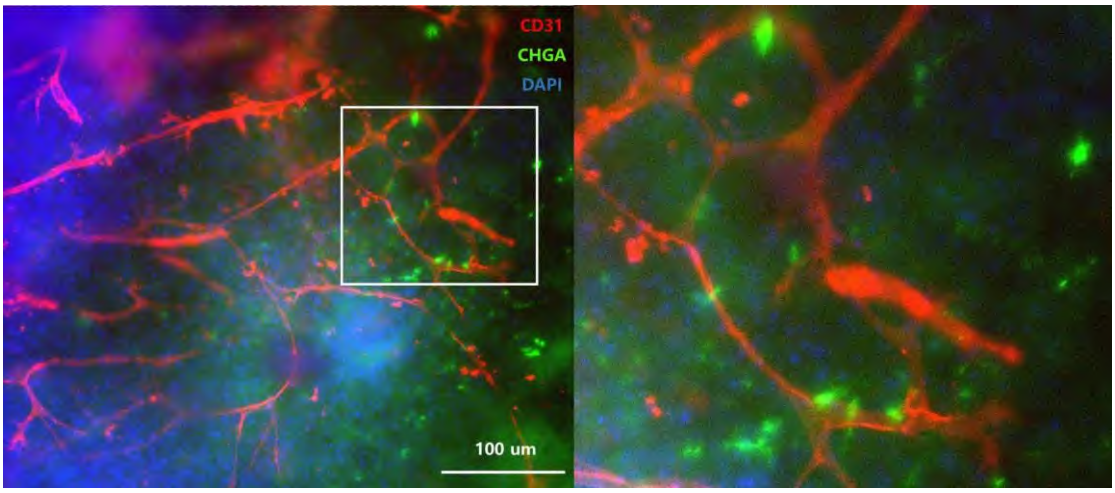

Fig. 5. Marker Expression

The intestinal organoids differentiated using the Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) show expression of enterochromaffin cells (CHGA) and endothelial cells (CD31).

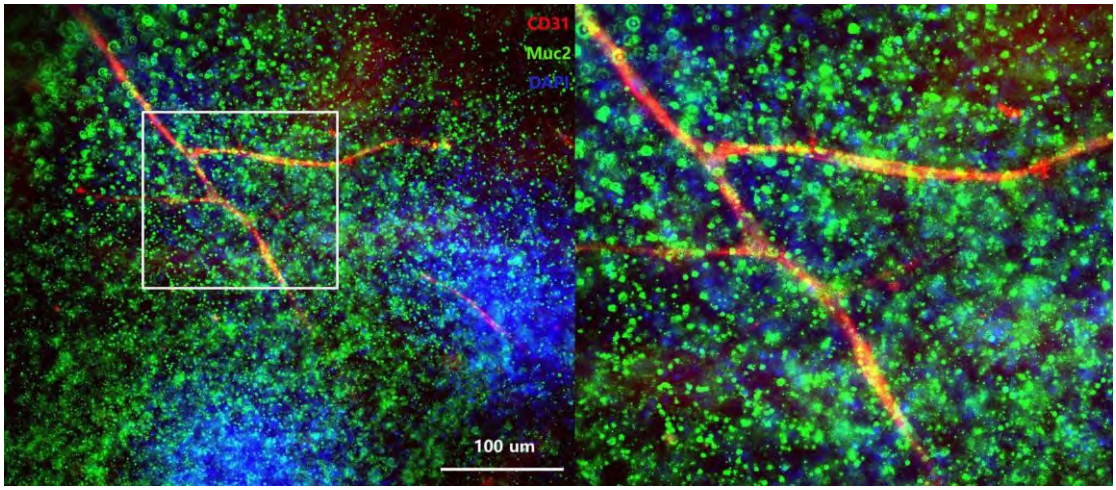

Fig. 6. Marker Expression

The intestinal organoids differentiated using the Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) show expression of goblet cells (mucus-producing, MUC2) and endothelial cells (CD31).

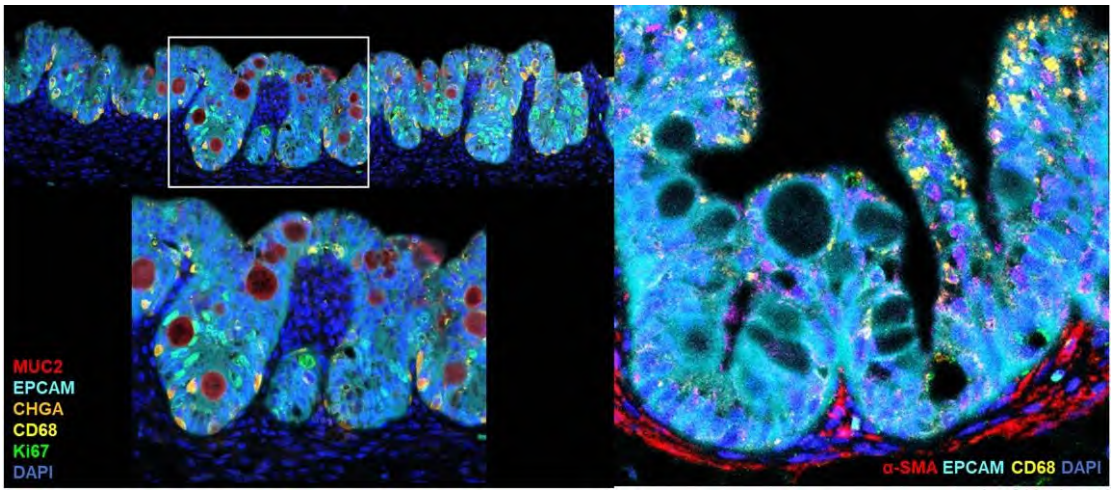

Fig. 7. Marker Expression

The intestinal organoids differentiated using the Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) show expression of smooth muscle cell (α-SMA); epithelial cell (EPCAM); macrophage (CD68); goblet cell (MUC2); Ki67 (intestinal stem cell) and enteroendocrine cells (CHGA).

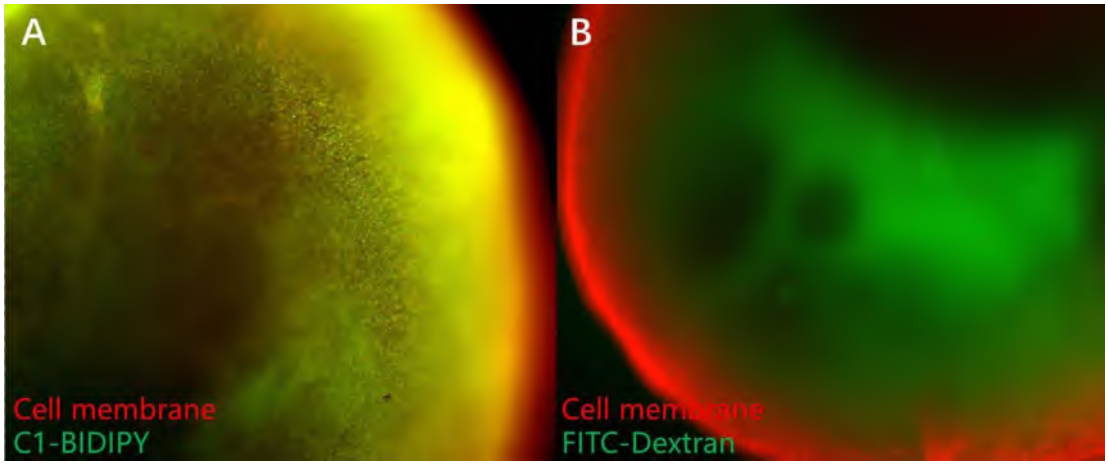

Fig. 8. Organoid Activity

Intestinal organoids differentiated using the Human iPSC-Derived Intestinal Organoid Differentiation Kit (Cat. No. RIPO-IWM005K) show normal intestinal function validated by the absorption of fatty acid and glucose.