

Human iPSC-Derived Intestinal Organoid Differentiation Kit

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Cat. No. : RIPO-IWM005K

Product Description

Human iPSC-Derived Intestinal Organoid Differentiation Kit (Ca. 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 intestinal regions. This kit can produce in minimum 48 intestinal organoids. Organoids generated using Human iPSC-Derived intestine Organoid Differentiation Kit (Ca. No. RIPO-IWM005K) feature various types of cells, including intestine epithelium cells, mesenchyme cells, enterocytes, Paneth cells, goblet cells, etc. These intestine organoids show intestine crypt like structure, Villi and Microvilli like structure. These intestine organoids show 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 years from date of manufacture (MFG) on label
Basal Medium B	RIPO-IWM005K-C01	13 ml	4°C	Stable for 1 years from date of manufacture (MFG) on label
Supplement B	RIPO-IWM005K-1-C02	2 ml	-20°C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium C	RIPO-IWM005K-C02	13 ml	4°C	Stable for 1 years from date of manufacture (MFG) on label
Supplement C	RIPO-IWM005K-1-C03	2 ml	-20°C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium D	RIPO-IWM005K-C03	100 ml	4°C	Stable for 1 years from date of manufacture (MFG) on label
Supplement D	RIPO-IWM005K-1-C04	10 ml	-20°C	Stable for 1 years 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 diameter)
- Hemocytometer
- Trypan blue

Equipment Required

- Incubator (37°C, 5% CO₂)
- Low-speed centrifuge with a swinging bucket rotor with an adaptor for plate holders
- Incubated shaker
- Biosafety cabinet

Protocol Diagram

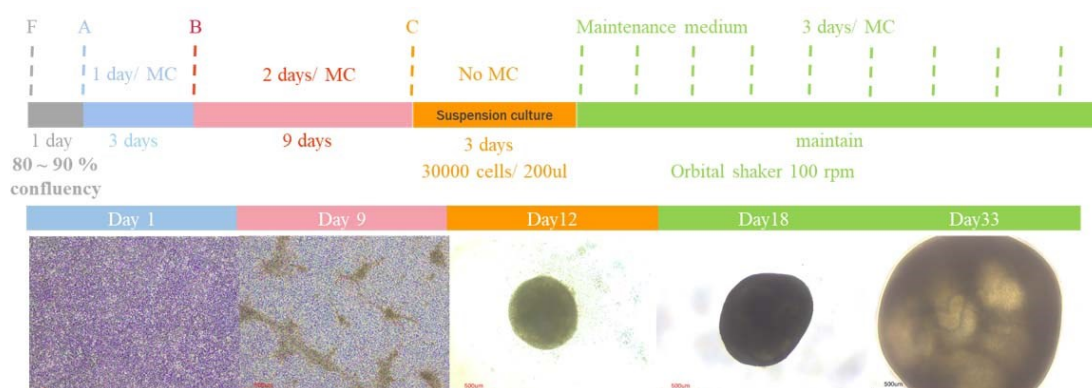


Figure 1. intestine Organoid Differentiation Process

The color differs each component of differentiation kit. The dashed line represents the time for medium changes. Morphology of intestine 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 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 C. 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 D. 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 your ipsc is cultured under the mTeSR-based culture system in a 6-well plate coated by Matrigel. The cell confluence should be 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.

Intestine Organoid Differentiation

1. Aspirate medium from hPSC culture and add 3 ml of medium A at the well and incubate at 37°C, 5% CO₂ for 72 h.
2. After 72 h, remove the 3 ml medium in the well, add 3 ml of medium B and incubate at 37°C, 5% CO₂ for 9 days. Change the medium B every other day.
3. After 9 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.

Note: Please collect the cell clusters while washing by 300 g-3 min-centrifuge. The collected cell clusters should be digested as well.

4. Aspirate PBS and add 2 mL of Gentle Cell Dissociation Reagent for each well.
5. 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.

6. 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
7. Remove the supernatant and add 2-3 ml medium C to resuspend cells.
8. Count cells using Trypan Blue and a hemocytometer.
9. Add appropriate volume of medium C to acquire final concentration of 50000 cells/ml
10. Add 200 µL of cell suspension into each well of a 96-well round-bottom ultra-low attachment plate (10000 cells/well).

11. Incubate the plate at 37°C, 5% CO₂. Centrifuge the ultra-low attachment plate at 300 g, for 3 minutes if the formation of sphere is not observed after 24 h. proceed to the incubation for another 48 h.
12. After the last day of incubation with medium C, transfer all intestine organoids into Ultra-low attachment 6 well plate with in maximum 24 organoids per well. Add 5 ml medium D per well. Put the plate on an orbital shaker, set the shaker at 100 rpm and put the shaker into incubation at 37°C, 5% CO₂ for 72 h.
13. Remove the 5 ml medium in each well, add 5 ml of medium D and incubate at 37°C, 5% CO₂ for 72 h.
14. Repeat step (13) 8 time (add 10 times of medium D in total)
15. After 45 days of differentiation, remove medium D, add 5 ml of medium M-M per well, keep the plate on the shaker and incubate at 37°C, 5% CO₂.
16. Full medium change of medium M-M every 3 days with the volume of 30 ml.

Related Products

Product	Cat. No.
Intestinal Organoid maintenance medium	RIPO-IWM006

Validation Data of Intestinal Organoids

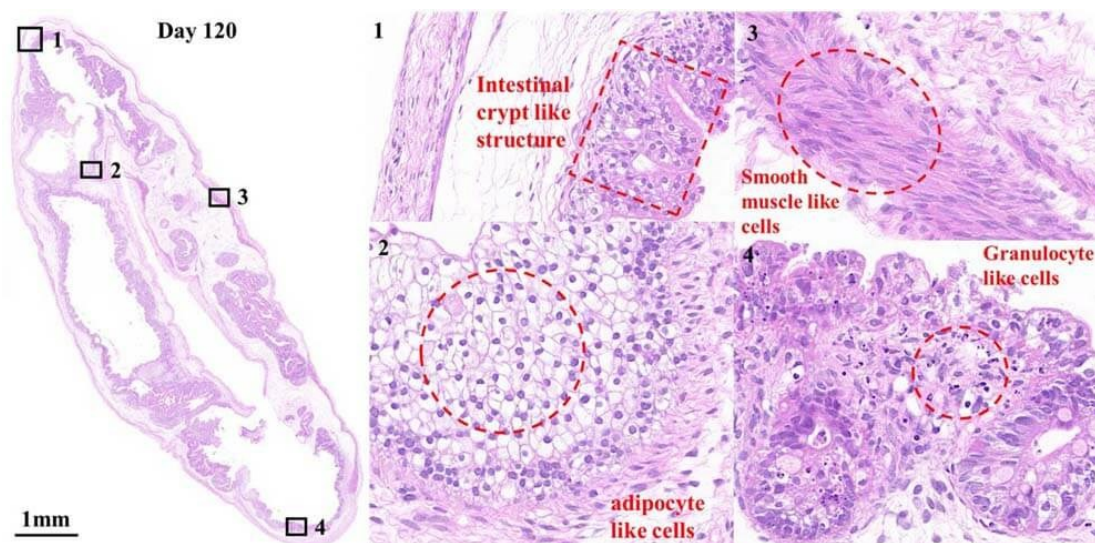


Fig 2. Organoid Histology

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

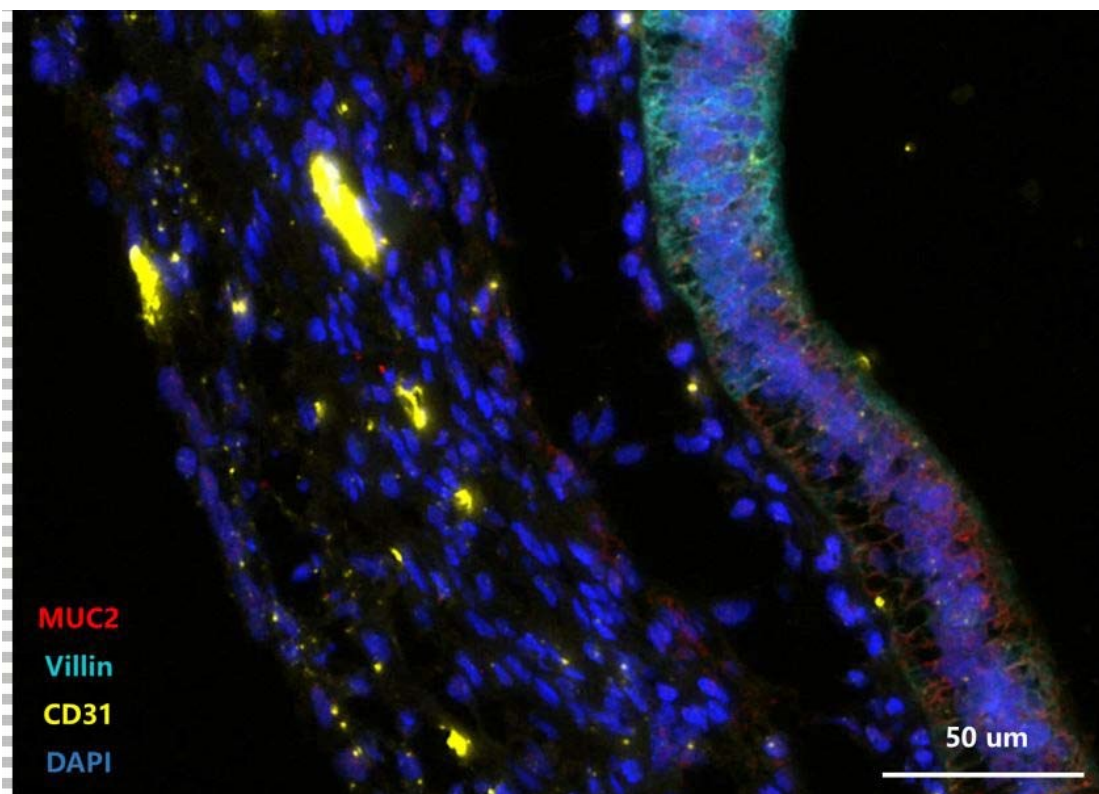


Fig 3. Marker expression

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

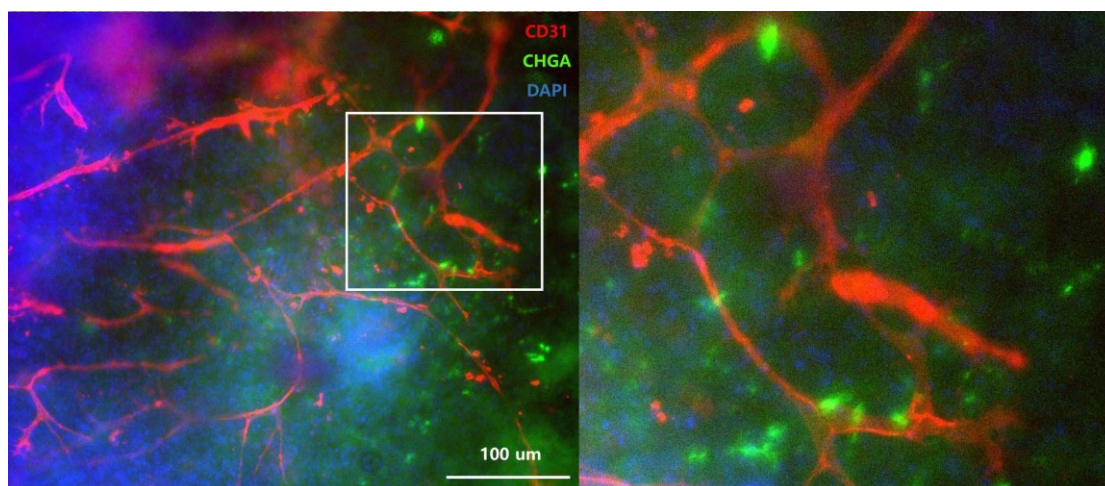


Fig 4. Marker expression

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

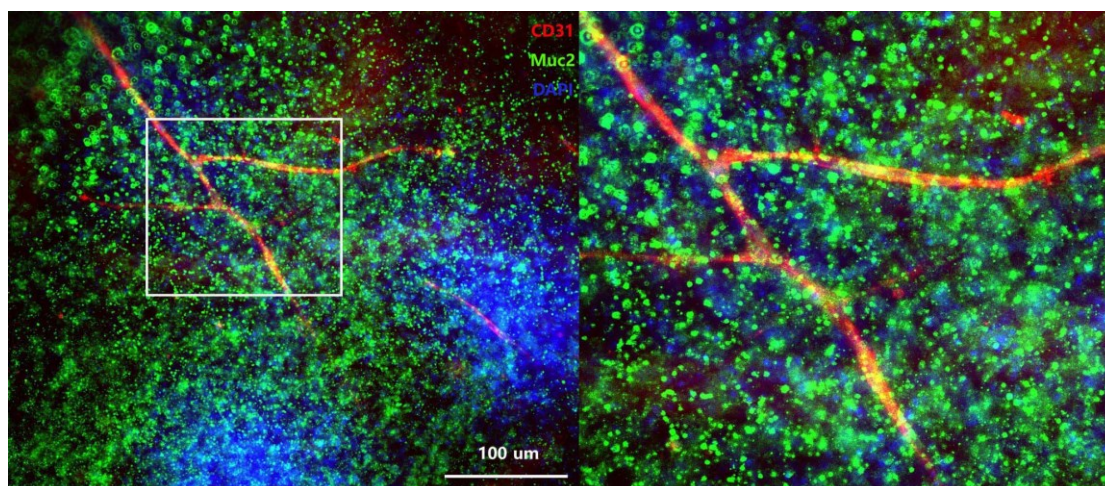


Fig 5. Marker expression

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

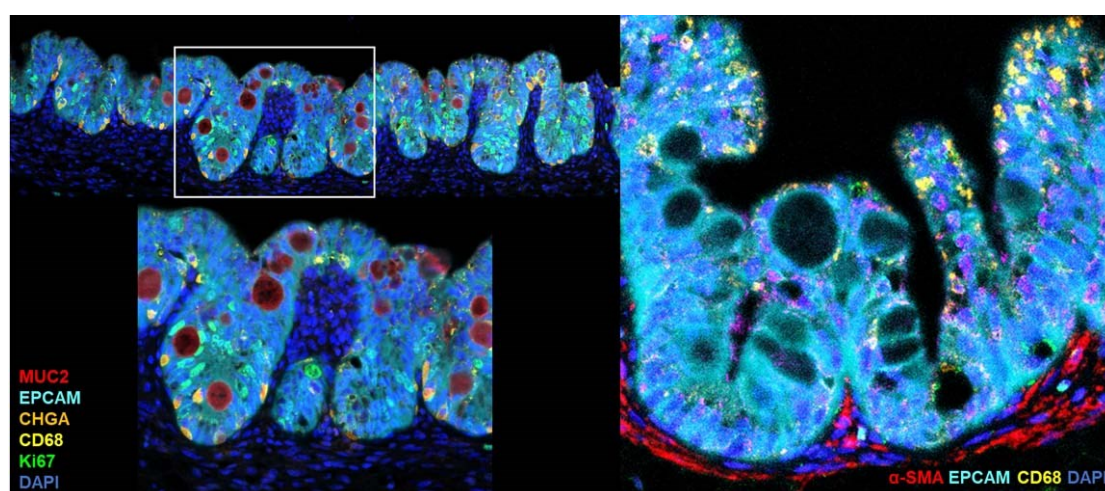


Fig 6. Marker expression

The intestine organoids differentiated using the Human iPSC-Derived intestine Organoid Differentiation Kit (Ca. 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).

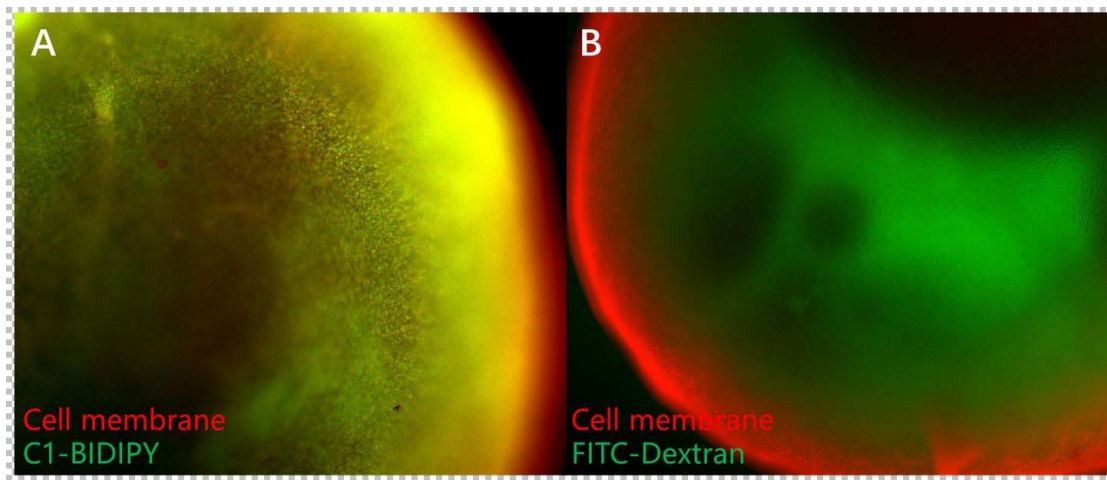


Fig 7. Organoid Activity

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