

# Human iPSC-Derived Intestinal Organoid differentiation Kit

## Human iPSC-Derived Intestinal Organoid Differentiation Kit

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. 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 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

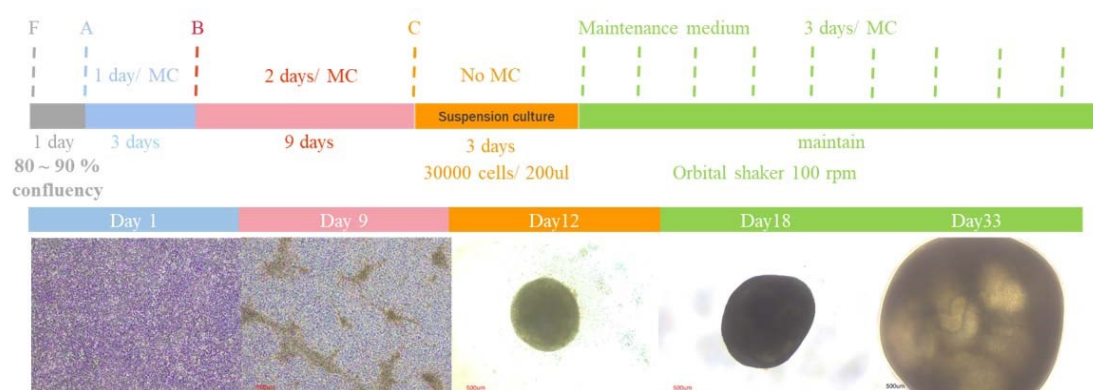
- mTeSR Plus (STEMCELL Technologies, # 100-0276)
- Gentle Cell Dissociation Reagent (STEMCELL Technologies, #100-0485)
- DMEM/F12 medium
- D-PBS (Without Ca++ and Mg++)
- Ultra-Low Attachment 96-well Plate
- Ultra-Low Attachment 6-well Plate
- Orbital shaker (any brand, 2 cm shaking diameter)

- Hemocytometer
- Trypan blue

## Equipment Required

- Incubator (37°C, 5% CO<sub>2</sub>)
- 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 intestine 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 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

### Induction

1. Aspirate medium from hPSC culture and add 3 ml of medium A at each well and incubate at 37°C, 5% CO<sub>2</sub> 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<sub>2</sub> 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 hPSC culture. Wash the well with 3 ml of pre-warmed D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>) 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 12ml of Gentle Cell Dissociation Reagent in the tube to resuspend the cell clusters.
5. Aspirate 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.

### 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 500000 cells/ml
11. Add 200 µl of cell suspension into each well of a 96-well round-bottom ultra-low attachment plate (100000 cells/well).
12. Incubate the plate at 37°C, 5% CO<sub>2</sub>. 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.
- After the last day of incubation with medium C, transfer all intestine organoids into Ultra-Low Attachment 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<sub>2</sub> for 72 h.
13. Remove the 5 ml medium in each well, add 5 ml of medium D and incubate at 37°C, 5% CO<sub>2</sub> for 72 h.
14. Change the medium by 5 ml of medium D and Repeat medium D change 8 time (add 10 times of medium D in total).

### Intestinal organoid maturation and maintenance

15. After 45 days of differentiation, remove medium D, add 5 ml of medium M-M and incubate at 37°C, 5% CO<sub>2</sub>.
16. Change the medium M-M fully every three days with the volume of 5 ml.

### Related Products

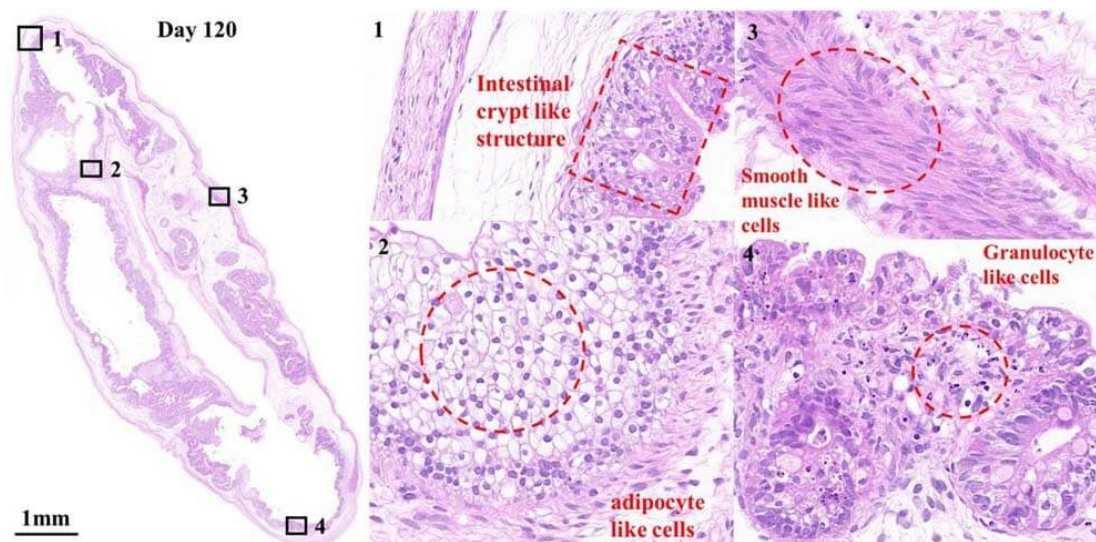
Product	Cat. No.
Intestinal Organoid maintenance medium	RIPO-IWM006

### Validation Data of Intestinal Organoids



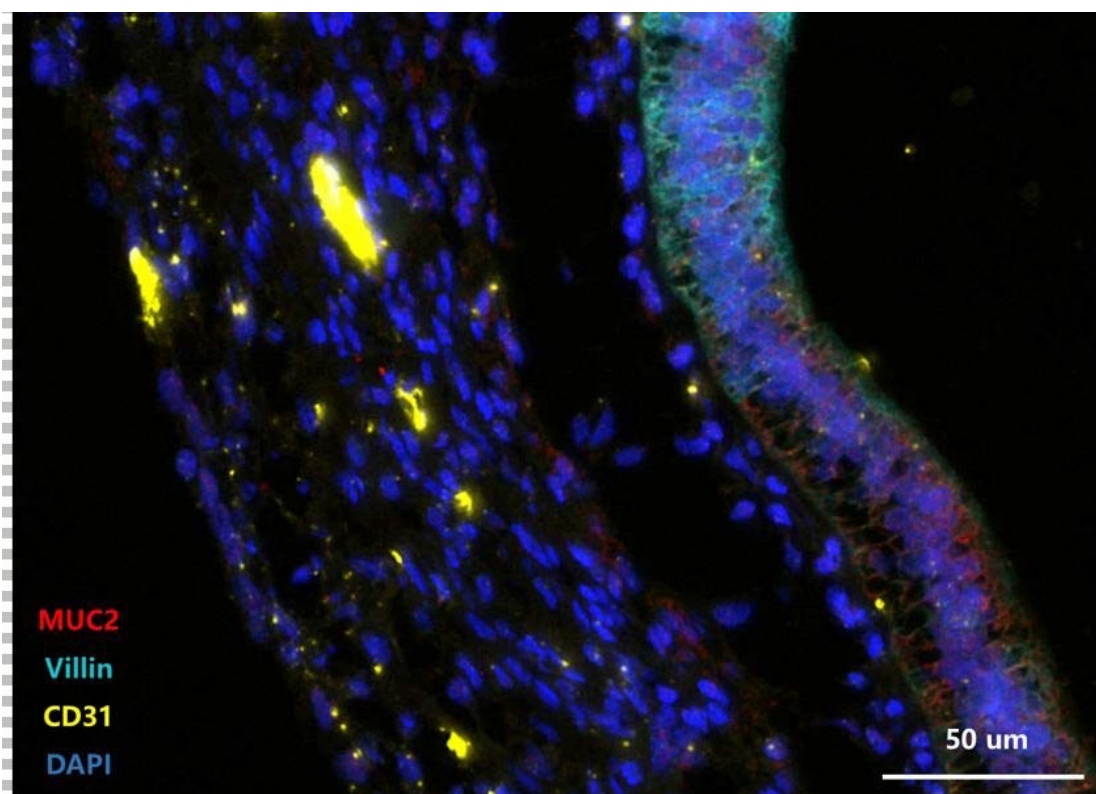
*Fig 2. Organoid morphology*

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



*Fig 3. Organoid Histology*

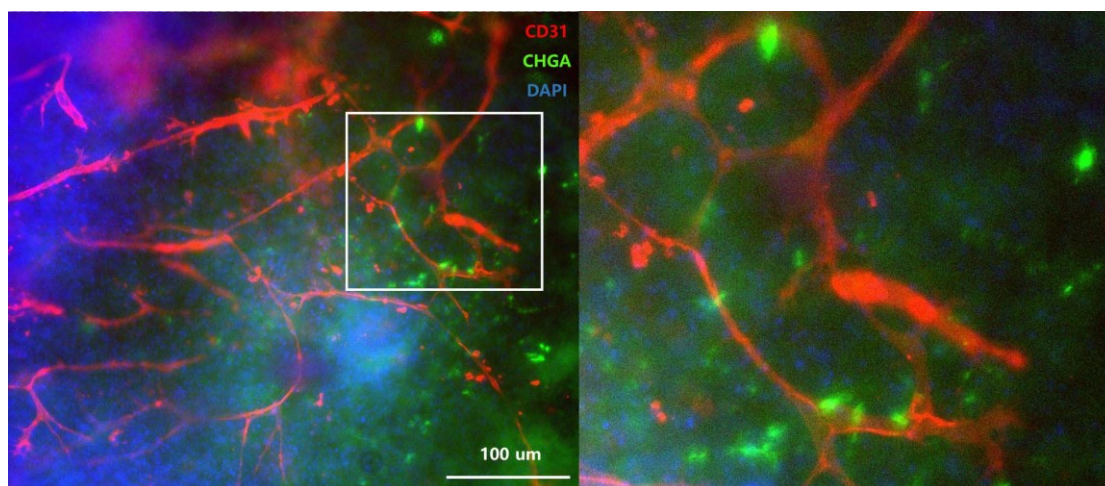
Observation of granulocyte-like cells, adipocyte like cells, smooth muscle like cells and intestinal crypt like structure by morphology on day 120 intestine organoids.



*Fig 4. Marker expression*

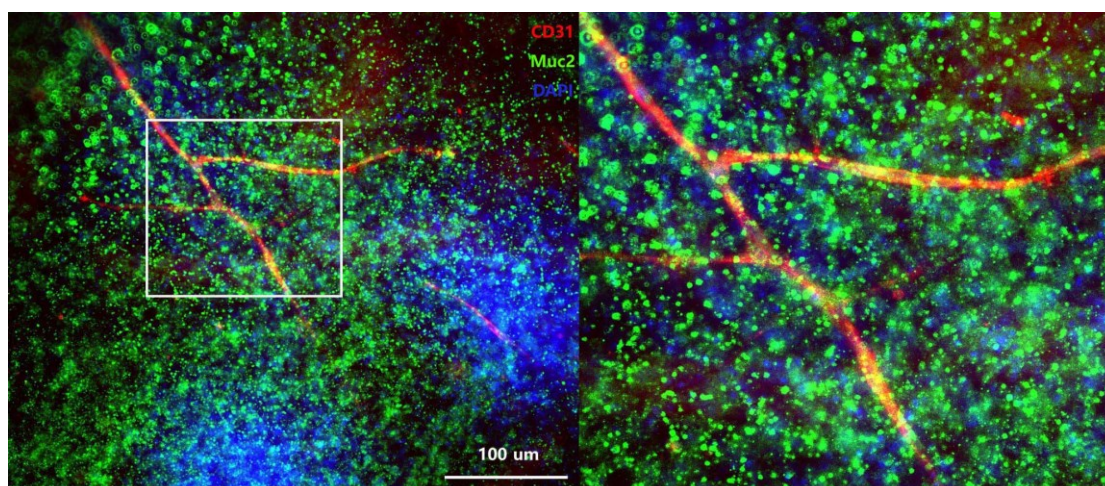
The intestine organoids differentiated using the Human iPSC-Derived intestine Organoid Differentiation Kit (Ca. No. RIPO-IWM005K) show expression of goblet cells (mucus-producing, MUC2), brush borders (Villin) and endothelial cells (CD31).





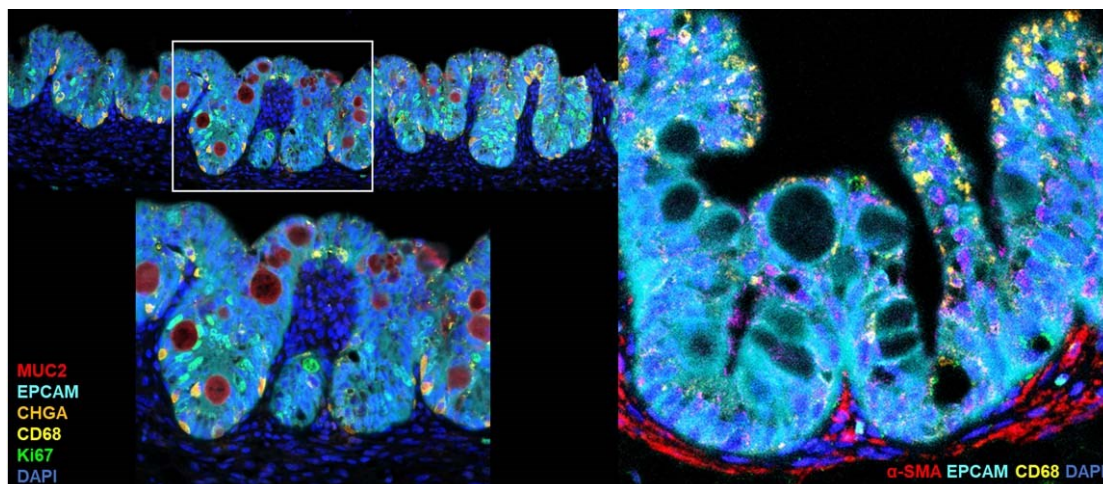
*Fig 5. Marker expression*

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).



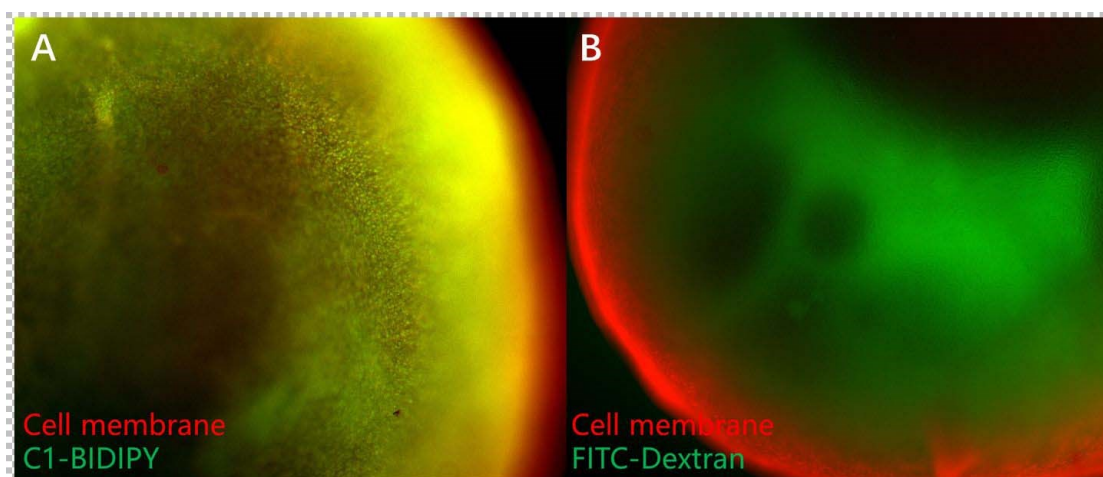
*Fig 6. Marker expression*

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 7. 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 ( $\alpha$ -SMA); epithelial cell (EPCAM); macrophage (CD68); goblet cell (MUC2); Ki67 (intestinal stem cell) and enteroendocrine cells (CHGA).



*Fig 8. 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.