

# Human iPSC-Derived Cardiac Organoid differentiation Kit

## Human iPSC-Derived Cardiac Organoid Differentiation Kit

Cat. No. : RIPO-HWM002K

### Product Description

Human iPSC-Derived Cardiac Organoid differentiation Kit (Ca. No.: RIPO-HWM002K) allows hESC or iPSC to differentiate into cardiac organoids. Cardiac organoids are three-dimensional *in vitro* models with a cellular composition and structural organization that is resembles to human heart. This kit can produce 24 cardiac organoids in four steps. Differentiation was carried out by forming embryoid bodies (EBs) from hPSC at an Ultra-Low Adherent 96 Well Plate, and then changing the medium according to the instructions. A spontaneously beating cardiac organoid with a cavity can be seen between day 6 and 13 of the onset of differentiation (the starting time of beating depends on cell lines). Cardiomyocytes, key cellular component of cardiac organoid, are responsible for the beating of the cardiac organoids. The beating rate of the formed cardiac organoids increases dramatically when stimulated with isoproterenol or forskolin, and there is a linear relationship between the concentration of the drug and the beating rate over a range of concentrations.

### 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 cardiac organoid differentiation.

### Product Information

Name	Component #	Size	Storage	Shelf Life
Medium F	RIPO-HWM002K-1-C01	3 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium A	RIPO-HWM002K-C01	5.4 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Supplement A	RIPO-HWM002K-1-C02	0.6 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium B	RIPO-HWM002K-C02	22.5 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Supplement B	RIPO-HWM002K-1-C03	2.5 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium C	RIPO-HWM002K-C03	27 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Supplement C	RIPO-HWM002K-1-C04	3 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label
Basal Medium M-M	RIPO-HWM002K-C04	22.5 ml	4°C	Stable for 1 year from date of manufacture (MFG) on label
Supplement M-M	RIPO-HWM002K-1-C05	2.5 ml	-20°C	Stable for 1 year from date of manufacture (MFG) on label

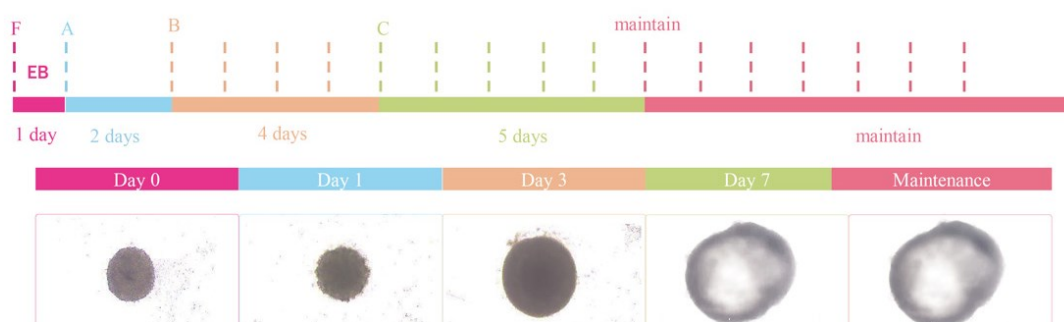
## Materials Required but Not Included

- mTeSR Plus (STEMCELL Technologies, # 100-0276)
- Gentle Cell Dissociation Reagent (STEMCELL Technologies, # 100-0485)
- D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>)
- Ultra-Low Attachment 96 Well Plate (Corning, #7007)
- Ultra-Low Attachment 6 Well Plate (Corning, #3471)
- 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
- Orbital shaker
- Biosafety cabinet

## Protocol Diagram



*Figure 1. Cardiac Organoid Differentiation Process*

The color differs each component of differentiation kit. The dashed line represents the time for medium changes. Morphology of cardiac 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 (6 ml)	Basal Medium A	5.4 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	0.6 ml	
Medium B (25 ml)	Basal Medium B	22.5 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	2.5 ml	
Medium C (30 ml)	Basal Medium C	27 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	3 ml	
Medium M-M (25 ml)	Basal Medium M-M	22.5 ml	Mix completely the Basal Medium M-M and Supplement M-M to get Medium M-M. Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement M-M	2.5 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 cardiac organoid culturing, please make sure that the culture system you use is mTeSR-based. 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.**

### EB Formation

1. Aspirate medium from hPSC culture and wash the well with 3 ml of pre-warmed D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>) 3 times.
2. Aspirate PBS and add 2 ml of Gentle Cell Dissociation Reagent.
3. Incubate about 7-10 minutes for digestion of iPSCs to single cells.

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

4. Using pipettes to pipet cells for obtaining single cells and centrifuge at 300 g, 4°C for 5 minutes
5. Remove the supernatant and add 1 ml mTeSR Plus to resuspend cells.
6. Count cells using Trypan Blue and a hemocytometer.
7. Transfer appropriate number of cells into Medium F to acquire final concentration of 7,500 cells/100 µl.
8. Add 100 µl of cell suspension into each well of a 96-well round-bottom ultra-low attachment plate.
9. Centrifuge the ultra-low attachment plate seeded with cells at 300 g, 4 °C for 5 minutes.
10. Incubate the plate at 37°C, 5% CO<sub>2</sub> for 24 h to form embryoid bodies.

11. Observe plate under microscope. The size of formed EBs should be ranging from 300 to 500  $\mu\text{m}$  and with a smooth round edge (see protocol diagram). Count this day as day 0.

### EB Validation

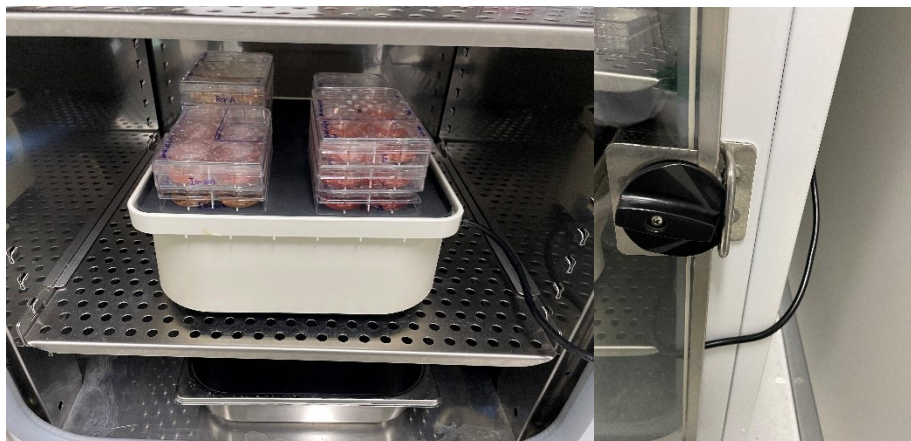
#### Morphology

- Round in shape with smooth edges and with limited dead cells surrounding.
- Diameter should be between 400-500  $\mu\text{m}$ .



### Cardiac Organoid Differentiation

1. At day 0, remove all the medium in the wells. Add 200  $\mu\text{l}$  of Medium A at each well and incubate at 37°C, 5% CO<sub>2</sub> for 48 h.
2. After 48 h, remove the 200  $\mu\text{l}$  medium in each, add 200  $\mu\text{l}$  of medium B in each well and incubate at 37°C, 5% CO<sub>2</sub> for 24 h.
3. Repeat step (2) 3 times (add 4 times of medium B in total)
4. After the last incubation with medium B, remove the 200  $\mu\text{l}$  of medium B, add 200  $\mu\text{l}$  of medium C and incubate at 37°C, 5% CO<sub>2</sub> for 24 h.
5. Repeat step (4) 4 times (add 5 times of medium C in total)
6. After the last incubation with medium C, transfer all cardiac organoids into ultra-low attachment 6-well plate (the maximum number is 24 organoids per well) and add 5 ml medium M-M per well. Then put the plate on an orbital shaker (as shown figures), which was placed inside the incubator, with the speed of 100 rpm.

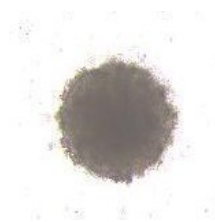


7. Change the medium M-M fully every three days with the volume of 5 ml.

### Differentiation Validation

#### Directly after stage A:

- The edge of EBs would change to irregular or smooth (Both could be accepted).
- Size is ranging from 400 to 500  $\mu\text{m}$ .



Stage B:

- After first 24 h incubation with medium B, the edge would change to smooth in shape.
- After 72 h incubation with medium B, the EBs would appear whole vacuum in the middle (If the shape changes to irregular and no vacuum appears, the differentiation is failed).
- The size after B stage would be larger than 800  $\mu\text{m}$ .

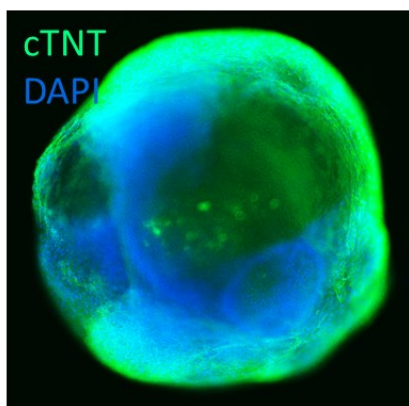


Beating:

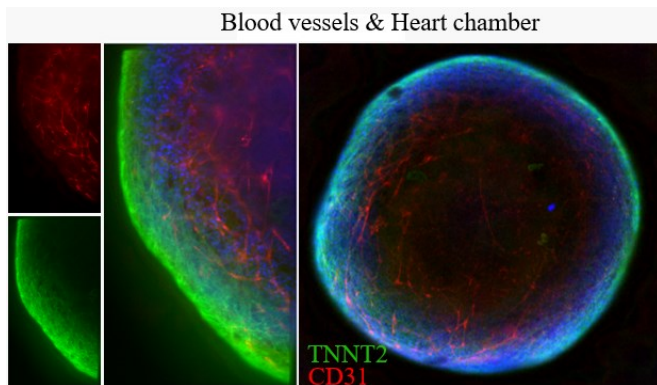
- Regular beating would appear at 6-12 days from EB formation.
- If no beating is observed even day 12 from differentiation, the differentiation could be considered as failed.

Marker expression (for maturated cardiac organoids)

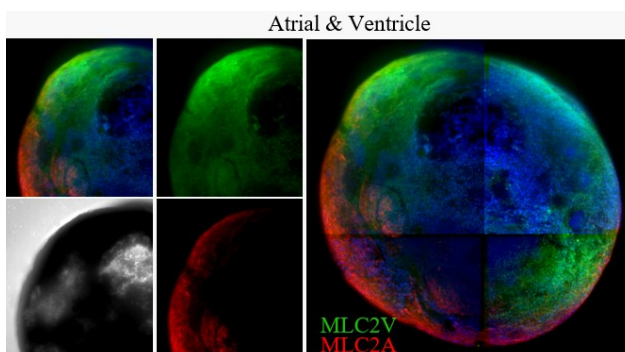
- Presence of cardiomyocytes: cTNT marker expression, which occupies almost 80% of total cells, is acceptable (Day 15).



- Presence of endothelia cell: CD31 (Day 25)



- Presence of ventricle and atrium chambers: MLC2V and MLC2A respectively (Day 10)



## Related Products

Product	Cat. No.
Cardiac Organoid maintenance medium	RIPO-HWM004