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	3ml	-20°C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium A	RIPO-HWM002K-C01	5.4ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement A	RIPO-HWM002K-1-C02	0.6ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium B	RIPO-HWM002K-C02	22.5ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement B	RIPO-HWM002K-1-C03	2.5ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium C	RIPO-HWM002K-C03	27ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement C	RIPO-HWM002K-1-C04	3ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium M-M	RIPO-HWM002K-C04	22.5ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement M-M	RIPO-HWM002K-1-C05	2.5ml	-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)
- D-PBS (Without Ca++ and Mg++)
- Ultra-Low Attachment 96 Well Plate (Corning, #7007)
- Ultra-Low Attachment 6 Well Plate (Corning, #3471)
- 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
- Orbital shaker
- Biosafety cabinet

Protocol Diagram

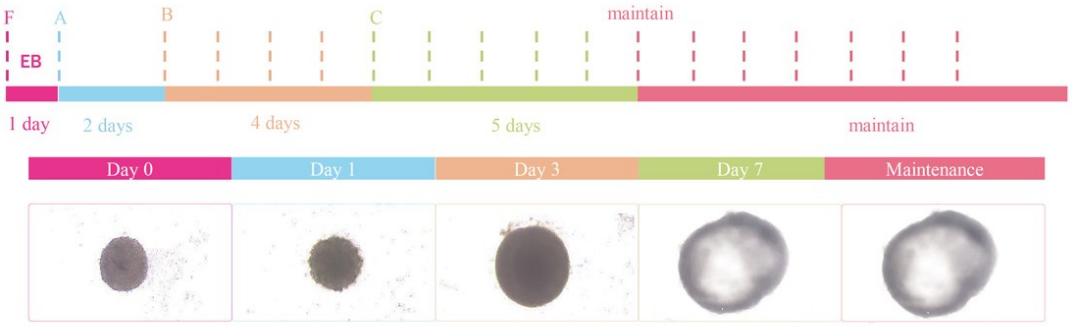


Figure 1. Cardiac Organoid Differentiation Process

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 (5ml)	Basal Medium A	5.4ml	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	0.6ml	
Medium B (20ml)	Basal Medium B	22.5ml	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	2.5ml	
Medium C (25ml)	Basal Medium C	27ml	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	3ml	
Medium M-M (50ml)	Basal Medium M-M	22.5ml	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	2.5ml	

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++ and Mg++) 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 300g, 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 300g, 4 °C for 5 minutes.
10. Incubate the plate at 37°C, 5% CO_2 for 24h to formation of embryoid bodies.

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

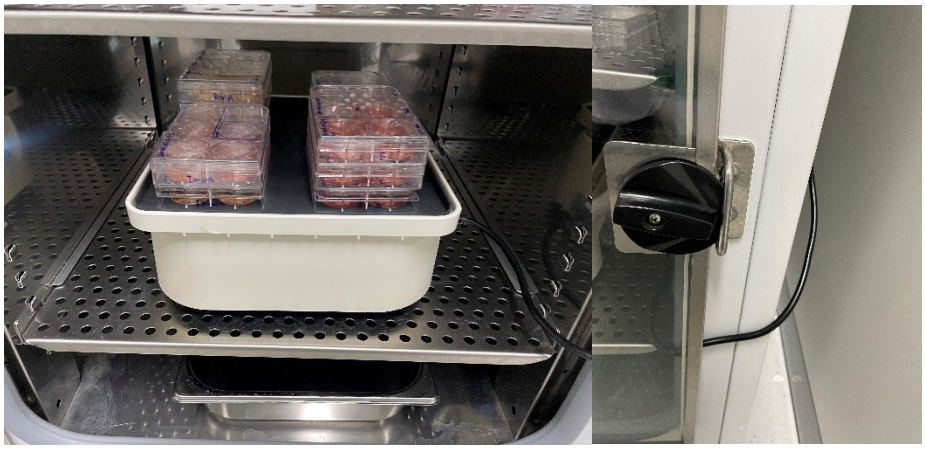
EB Validation

Morphology

- Round in shape with smooth edges and with limited dead cells surrounding.
- Diameter should between 400-500 um.

Cardiac Organoid Differentiation

1. At day 0, remove all the medium in the wells. Add 200µl of Medium A at each well and incubate at 37°C, 5% CO_2 for 48h.
2. After 48h, remove the 200µl medium in each, add 200µl of medium B in each well and incubate at 37°C, 5% CO_2 for 24h.
3. Repeat step (2) 3 time (add 4 times of medium B in total)
4. After the last incubation with medium B, remove the 200µl of medium B, add 200µl of medium C and incubate at 37°C, 5% CO_2 for 24h.
5. Repeat step (4) 4 time (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.

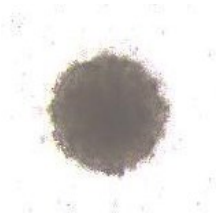


7. Change the medium M-M fully every other day with the volume of 5 ml.

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 um.



Stage B:

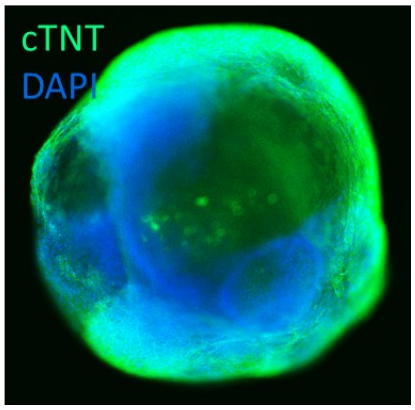
- After first 24 h incubation with medium B, the edge would change to smooth in shape.
- Afte 72 h incubation with medium B, the EBs would appear whole vacuum in the middle (If the shape changes to irregular and no vacuum appears, the differentiation is failed).
- The size after B stage would be larger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

Beating:

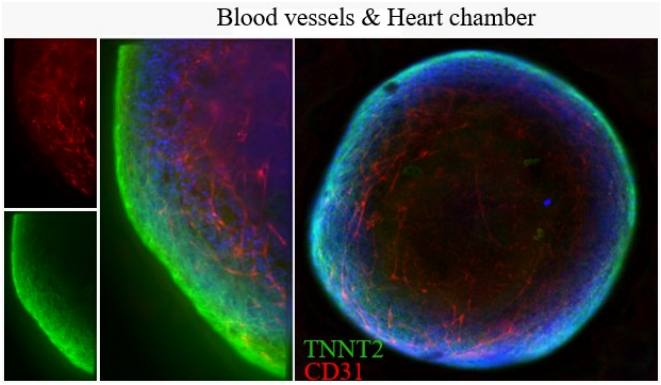
- Regular beating would appear at 6- 12 days from EB formation.
- If no beating is observed even day 12 from differentiation, the differentiation could be considered as failed.

Marker expression (for maturated cardiac organoids)

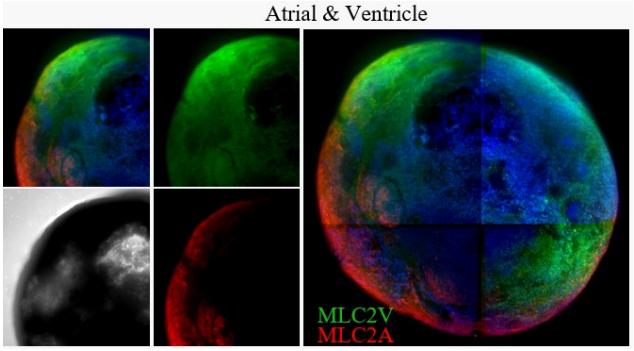
- Presence of cardiomyocytes: cTNT marker expression, which occupies almost 80 % of total cells, is acceptable (Day 15).



- 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**

Validation Data of Cardiac Organoids

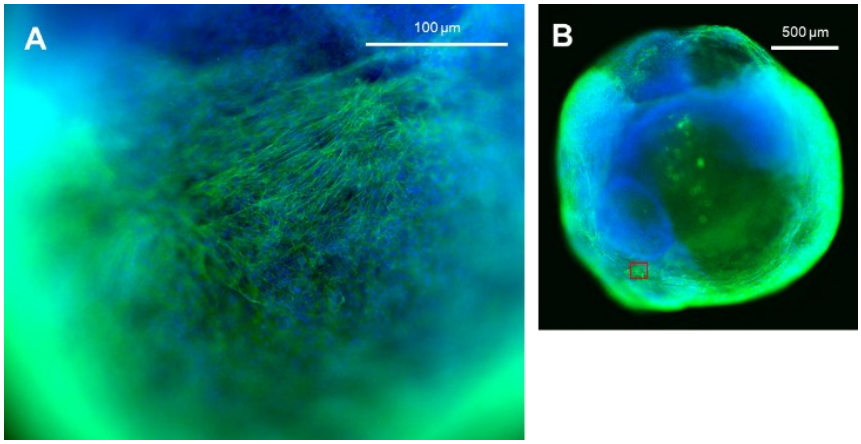


Figure 2. Immunostaining of Cardiac Organoids

(A) 20x view of cardiac organoids with cardiomyocytes were visualized using the cTNT marker with nuclei visualized by DAPI. (B) Presence of cardiomyocytes throughout the entire organoid can be observed.

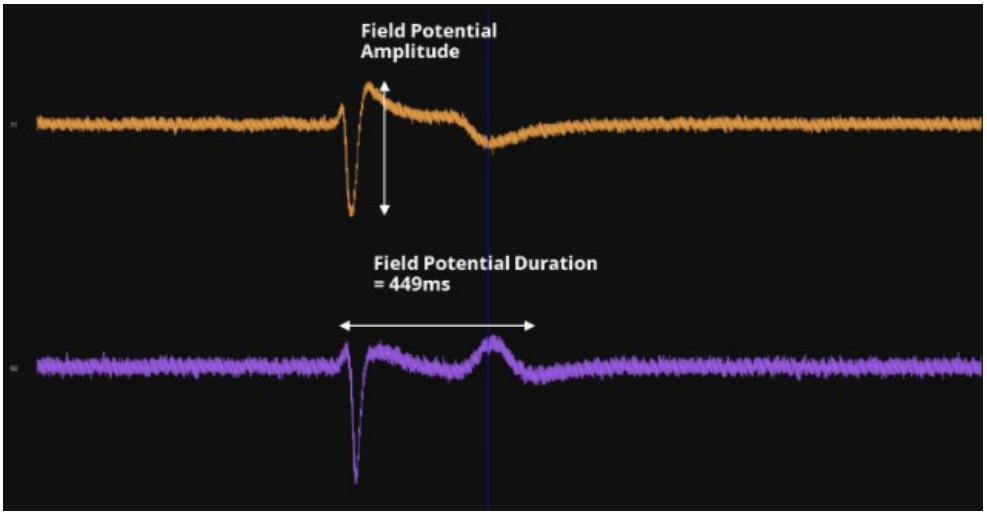


Figure 3. Transient Voltage Potential across Cardiac Organoids

Silicon probes were placed across a cardiac organoid to measure the impulse generation and propagation causing the contraction activity. Each contraction lasts around 449 ms and can be continuously observed.

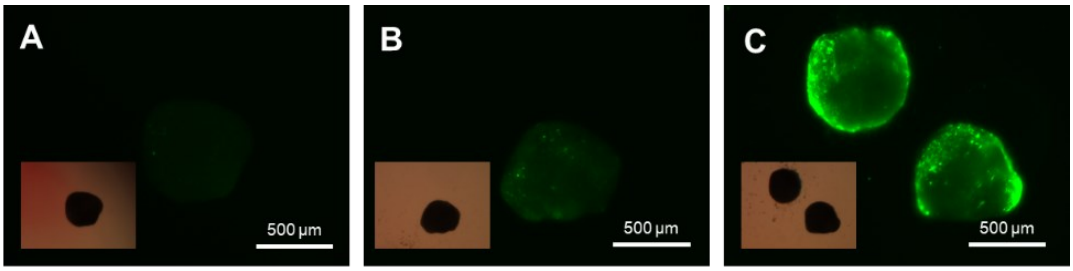


Figure 4. AAV Capsid Screening by Cardiac Organoids

Cardiac organoids grown for 11 days were infected with (A) AAV5-WT, (B) IVB-1, (C) IVB-2. Each of them was individually placed into a well plate. Transgene delivery efficacy was visualized by fluorescent intensity from GFP transgene expression.