

Human iPSC-Derived Cardiac Organoid Maintenance Kit

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Cat. No.: RIPO-HWM004

Product Description

Cardiac organoids are three-dimensional in vitro models with a cellular composition and structural organization that is representative of the human heart. Human iPSC-Derived Cardiac Organoid maintenance Kit allows the maturation and the long-term maintenance of cardiac organoids.

Product Specification

The basic medium of this 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
Cardiac Basal Medium M-M	RIPO-HWM004-C01	225ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Cardiac Supplement M-M	RIPO-HWM004-1-C01	25ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label

Materials Required but Not Included

- Ultra-Low Attachment 96 Well Plate
- · Ultra-Low Attachment 6 Well Plate
- · Orbital shaker (any brand, 2 cm shaking dimeter)

Equipment Required

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



Protocol Diagram

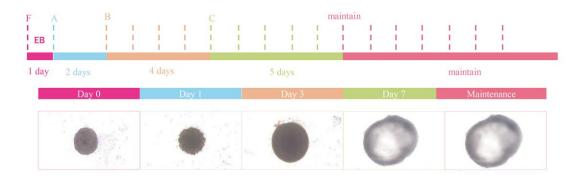


Figure 1. Cardiac Organoid Differentiation Process

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
	Basal Medium M-M	225ml	Mix completely the Cardiac Basal
Cardiac Medium M-M (500ml)	Supplement M-M	25ml	Medium M-M and Cardiac Supplement M-M to get Cardiac Medium M-M. Store at 2 - 8°C for up to 2 weeks or aliquot as desired.

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: This kit only serves for the Maturation and Maintenance of cardiac organoids. For the differentiation of cardiac organoid, please use Human iPSC-Derived Cardiac Organoid Differentiation Kit (RIPO-HWM002K).

Cardiac organoid maintenance

Case A: If you are using this kit following the differentiation kit.

- 1. Make sure that all cardiac organoids are transferred into ultra-low attachment 6 well plate (the maximum number is 24 organoids per well).
- 2. Make sure the plates are placed on an orbital shaker (as shown figures), which was placed



inside the incubator (37 $^{\circ}$ C, 5% CO₂), with the speed of 100 rpm.

3. Aspirate all medium in the wells and add 5 ml medium M-M per well.



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

Case B: If you are using this kit for purchased live cardiac organoids.

- 1. Make sure the cardiac organoids are recovered for 48 hours with the recovery medium delivered with the live organoids.
- 2. Make sure that all cardiac organoids are transferred into ultra-low attachment 6 well plate (the maximum number is 24 organoids per well).
- 3. Make sure the plates are placed on an orbital shaker (as shown figures), which was placed inside the incubator (37 °C, 5% CO₂), with the speed of 100 rpm.
- 4. Aspirate all medium in the wells and add 5 ml **medium M-M** per well.



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

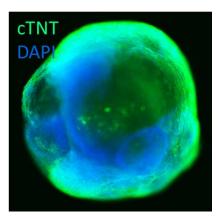
Validation

The cardiac organoids should show cavity-like structure and have regular beating.

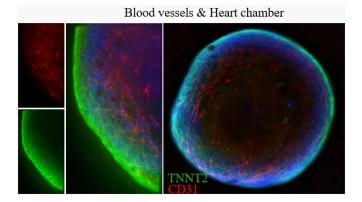
Marker expression (for maturated cardiac organoids)

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

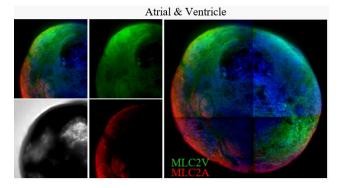




• Presence of endothelia cell: CD31 (Day 25)



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



Related Products

Product	Cat. No.
Human iPSC-Derived Cardiac Organoid Differentiation Kit	RIPO-HWM002K
Ready-to-use Human iPSC-Derived Cardiac Organoids	CIPO-HWL002K