COSMO BIO CO., LTD.
Inspiration for Life Science

For research use only. Not for clinical diagnosis.

Catalog No. PMC-CMC12-COS

Cardiomyocyte Culture Kit (Mouse)

(Mouse Cryopreserved Cell and culture medium)

Principle

Cardiomyocyte is one of the cell groups that compose heart. Cardiomyocyte is known as beating involuntary striated muscle cells. The beat that is the feature of the cardiomyocyte can be widely used for a pharmacologic and electrophysiology assays, because it responds to a variety of stimuli including hormones, drugs and electricity.
Cardiomyocyte Culture Kit contains cryopreserved cardiomyocytes, culture medium and fibronectin. Cells are isolated from mouse embryos' heart, and cardiomyocytes are enriched by removal of non-cardiomyocytes. The involuntary beat of the cells can be seen in culture using Cardiomyocyte Culture Kit (PMC-CMC12-COS).

Components

Product Name / Code No.	Size	Quantity	Storage Conditions	Shelf Life
Cardiomyocyte (Mouse)	2 x 10^6 cells/vial (0.5mL/vial)	1	Liquid Nitrogen	1 year
Culture Medium (Code: PMC-CMCM-COS)	125 ml	1	-20°C Freezer	6 months
Fibronectin	12 ml	1		

Shipping: dry ice

Components of Media:

PMC-CMCM-COS is optimized for the in vitro growth of cardiomyocytes. It is a liquid basal medium (D-MEM/F-12) containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, newborn calf serum, and antibiotics.

Materials required but not provided

- Variable volume pipettes
- Fibronectin-coated 24-well culture plate (flat bottom)

Precautions

1. Read the instructions carefully before beginning the culture.
2. This kit is for research use only, not for human or diagnostic use.
3. Upon receiving, cryovials are Immediately transferred to and stored in liquid nitrogen until ready to use. It is recommended to use cell vial immediately upon receiving.

Protocols

<1-1. Cultured with the 24well-plate>

A seeding density of 2.0×10^5 cells/well is recommended.

1. Thaw the Culture medium in at 37°C water bath with gentle shaking.
2. Quickly thaw the Cardiomyocyte vial for 1min 15sec in a 37°C water bath.
3. Transfer the vial contents of thawed cells into a 15 ml centrifuge tube containing 4.5 ml of Culture Medium.
4. Gently mix gently the cell suspension by slow pipetting up and down, and adjust cell density to 2.0×10^6 cells/5 ml solution in the tube.
5. Transfer 0.5 ml of cell suspension to each well of fibronectin-coated 24-well plate.
6. Incubate the plate at 37°C in a 5 % CO_2 humidified incubator.
7. The next day, gently add 0.5 ml of fresh pre-warmed culture medium to each well.
8. Exchange the medium with fresh and pre-warmed culture medium every day or every other day until the culture reaches 80-100% of cell confluent.

   * Culture reaches its confluent within 3-5 days, and cardiomyocytes begin beating.
   * Do NOT use cold culture medium. Please pre-warm culture medium at 37°C before use to ensure the viability of cardiomyocytes.

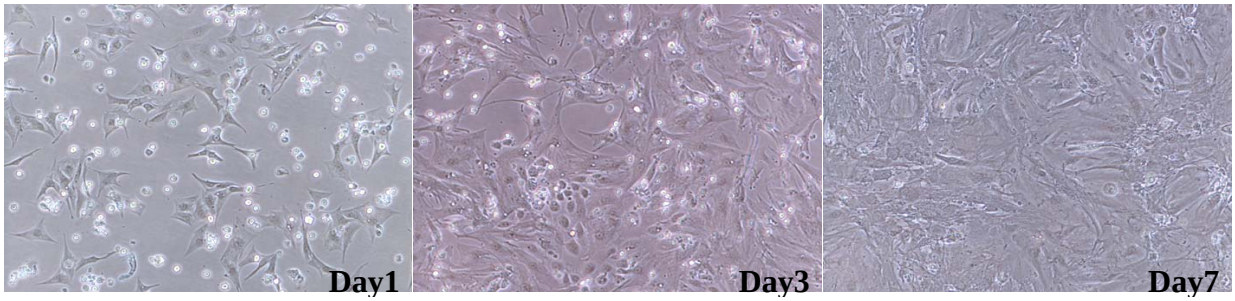

Fig. 1 Morphology of cardiomyocyte

<1-2. Culture adding 5-Bromo-2'-deoxyuridine>

It is recommended to supplement 5-Bromo-2'-deoxyuridine (BrdU) to culture in order to increase the purity of cardiomyocytes (BrdU prevents non-cardiomyocytes from developing). Preparation of BrdU: Dissolve BrdU (Sigma Cat. No. B-9285 or the similar product) in culture medium to a concentration of 10mM and filter sterilization. Fresh BrdU solution should be used for cardiomyocyte culture.

Prepare cell suspension as described above in steps 1-4 (<1-1. Cultured with the 24well-plate>).
Transfer 0.5 ml of cell suspension to each well of fibronectin-coated 24-well plate and add 5 µl of 10mM BrdU to each well (0.1 mM BrdU-final concentration).
Incubate the plate at 37°C in a 5 % CO_2 humidified incubator.
The next day, exchange the medium with fresh 0.5 ml of the medium containing 0.1 mM BrdU.
On day 3 of the culture, replace the medium with fresh 0.5 ml of the medium without BrdU.

< Prepare a fibronectin coated Culture 24-well plate >

Thaw fibronectin solution.
Add 0.5 ml of fibronectin solution to each well. (Add 0.25 ml/cm_2 of solution to each well in other types of culture plates.)
Leave the plate at 37°C in 5% CO_2 humidified incubation overnight.
Aspirate remaining material and rinse plates with sterile water twice. Plates are ready for use.

References

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