Cardiomyocyte Differentiation Media

CARDIO.D.Media-100

Media Usage Protocol:

Cardiomyocyte Differentiation Media is designed to be used with Murine Embryonic Stem Cells (not supplied by CET), Human Adipose Derived Mesenchymal Stem Cells, Human Amniotic Mesenchymal Stem Cells, Human Bone Marrow Derived Mesenchymal Stem Cells, Human Wharton's Jelly Derived Mesenchymal Stem Cells, Murine Adipose Derived Mesenchymal Stem Cells, Murine Bone Marrow Derived Mesenchymal Stem Cells, Rat Adipose Derived Mesenchymal Stem Cells and Rat Bone Marrow Derived Mesenchymal Stem Cells. When used as directed, over a period of 28 days, this media will fuse mesenchymal stem cells and form myotubes, which express cardiac proteins. When used with murine embryonic stem cells, this media will create beating cardiac colonies from embryoid bodies.

Note: Once complete media has been formulated, it should be stored at 4°C. Avoid extended exposure of the media to room or higher temperatures. Media should be equilibrated in a water bath set at 37°C before adding to any cell culture.

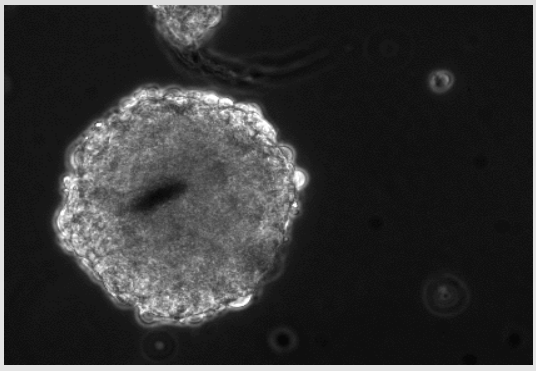

Figure 1: Cardiomyocyte Differentiation Media

Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.

Additional Reagents Needed

1. Fetal Bovine Serum, High Grade or Characterized. Store in aliquots of 50mL at -20°C.
2. Penicillin/Streptomycin/Amphotericin B solution, 100X or Penicillin/Streptomycin solution, 100X. These solutions should be portioned in 5mL aliquots, stored at -20°C and never freeze/thawed. Although anti-mycotics are not absolutely necessary, CET highly recommends their usage for long term cell culture.
3. You will need an active culture of the above mentioned cells at 70% confluency. Cells should be in logarithmic growth and should be touching each other for efficient cell to cell fusion. If you plan to use the media with murine embryonic cells, fresh embryoid bodies work the best for functional cardiomyocyte conversion.

CET has no recommendation as far as a specific vendor for these products but urges investigators to use the highest grade of reagents available for best results.

Formulating Complete Cardiomyocyte Differentiation Media

1. Defrost 5mL of fetal bovine serum and 1mL of antibiotic/antimycotic solution in a 37°C water bath until ice in the tubes is no longer visible.
2. Immediately disinfect the tubes and the bottle containing the base media with 70% isopropranol.
3. Working in a laminar flow hood, remove 6mL of the media from the bottle and discard. This and all other procedures must be done in a sterile manner.
4. Add 5mL of the fetal bovine serum to the base media.
5. Add 1mL of the antibiotic/antimycotic solution to the base media.
6. Cap the bottle containing the now complete media and gently swirl a few times. The complete media is now ready to use.
7. For any cell based applications, pre-warm the complete media to 37°C before use. Store complete media at 4°C when not in use.
8. As a general rule, cells should be fed with fresh, complete media every 72 hours and old media should be discarded before new complete media is added. Cardiomyocyte differentiation takes between 21 and 28 days on average depending on cell density.

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Certificate of Analysis
All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

Table 1. Preparation of 100mL Cardiomyocyte Differentiation Media

Brand	Amount For 500mL	Product	Catalog #
CET	94mL	CET Lung Cardiomyocyte Differentiation Media	CARDIO.D.Media-100
Any	5mL	Fetal Bovine Serum	Refer to Manufacturer's Catalog Number

Store media at 4°C.

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