iPS Cardiomyocyte Differentiation Media

CET.DIFF.CMM-100

CET's Cardiomyocyte (CM) Differentiation Media is designed to convert human iPSC into beating cardiomyocytes. CET can only guarantee beating cardiomyocytes using CET iPS lines (available separately). CET's CM Differentiation Media protocol is a multi-step process that is reliable and economical. By following this protocol, foci of beating cardiomyocytes can be generated. These can then be enriched using either flow cytometry or metabolic selection (e.g. glucose deprivation and use of sodium lactate).

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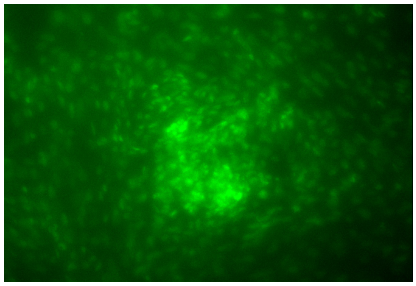

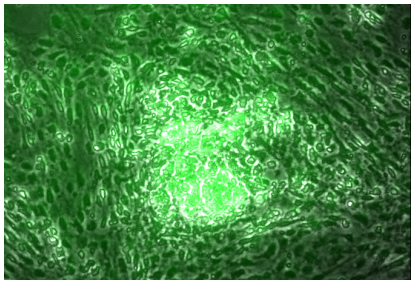

Note: Once complete media has been formulated, it should be stored at 4°C. Shelf life of complete media is 3 weeks. Avoid extended exposure of the medium to room temperature or higher temperatures. Medium should be equilibrated at room temperature before addition to any cell culture.

Coating Protocol (Day -1):

1. A day prior to plating your IPSC (both normal and disease specific IPS lines are available separately from CET), tissue culture dishes or plates must be coated with a thin layer of Vitronectin XF. Although there are various vendors of Vitronectin XF, CET recommends Stemcell Technologies Vitronectin XF. Coat dishes or plates according to manufacturer's directions. Those can be found at: https://www.stemcell.com/~/media/Technical%20Resources/B/5/F/0/A/29285PIS.pdf?la=en

iPSC Culture (Day 0-2):

Passage actively growing IPS colonies (with limited differentiation of less than 5%) onto Vitronectin XF coated plates. **Never plate frozen and thawed IPS cells directly onto Vitronectin XF coated surfaces.**

1. To passage iPSC, wash iPSC with pre-warmed 1X Dulbecco's Phosphate Buffered Saline (DPBS). Gently aspirate and add room temperature 1X Versene. Leave tissue culture plate or dish in a laminar flow hood at room temperature. It is not necessary to reintroduce the dish into a 37°C incubator.

2. At the end of 10 minutes, gently aspirate the Versene. Some cells will dislodge and be aspirated; this is normal. Add CET's iPS Growth Media without allowing the well to dry. Gently triturate the cells using a 5 mL serological pipette to create cell clumps. Cell clumps should consist of approximately 100-200 cells. Do not be too vigorous in this process since single cells will not re-plate. **OPTIONAL**: During initial passage of cells onto the Vitronectin XF coated surface, 5 micromolar Y-27632 can be added to CET's iPS Growth Media. If this is done, it must be withdrawn after the first 24 hours. **CET strongly recommends against single cell passaging using Dispase or Accutase since these tend to damage cells and greatly affects their replating efficiency.**

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3. Let IPS clumps attach in a 37°C incubator, without disturbing, for 24 hours. Feed with fresh CET iPS Growth media at the end of 24 hours. If you used Y-27632 during passaging, make sure this media does not contain this Rho Kinase inhibitor.

4. Depending on the iPS line that you are growing, they may have different proliferation or growth rates. However, it is critical to achieve 85% to 90% confluency on a given plate surface before proceeding to the differentiation process.

Differentiation into Cardiomyocytes (Day 2-4): Step 1

1. Reconstitute complete CET's Cardiomyocyte Differentiation Media Step 1 by thawing CET's Cardiomyocyte Differentiation Media Step 1 Supplement overnight at 4oC (in a refrigerator). **Never thaw supplement by placing it into a 37°C waterbath.** Add the entire contents of the supplement (2 milliliters) to CET's Cardiomyocyte Differentiation Media Step 1. Mix gently. Label, date and place complete Cardiomyocyte Differentiation Media Step 1 in a 4°C refrigerator. This media has a shelf life of 3-weeks if stored at 4°C.

2. Pre-warm CET's Cardiomyocyte Differentiation Media Step 1 to room temperature. **Do not place it into a 37°C waterbath.** Only aliquot the amount necessary to conduct a complete media replacement. CET recommends 200 microliters for a single well of a 96-well plate and 2 milliliters for a single well of a 6-well plate.

3. Gently aspirate the iPSC growth media from the tissue culture dish and introduce CET's Cardiomyocyte Differentiation Media Step 1. Place tissue culture dish back into the 37°C incubator.

4. Let cells incubate at 37°C for 2 days (48 hours) undisturbed.

Differentiation into Cardiomyocytes (Day 4-5): Step 2

1. At the end of this 48 hours (See Step 1, Point 4), gently aspirate Step 1 media. Introduce room temperature Cardiomyocyte Differentiation Media Step 2 and 4. To reconstitute complete CET's Cardiomyocyte Differentiation Media Step 2 and 4, thaw CET's Cardiomyocyte Differentiation Media Step 2 and 4 Supplement overnight at 4°C (in a refrigerator). **Never thaw supplement by placing it into a 37°C waterbath.** Add the entire contents of the supplement (2 milliliters) to CET's Cardiomyocyte Differentiation Media Step 2 and 4. Mix gently. Label, date and place complete Cardiomyocyte Differentiation Media Step 2 and 4 in a 4°C refrigerator. This media has a shelf life of 3-weeks if stored at 4°C.

2. Let cells incubate at 37°C for 1 days (24 hours) undisturbed.

Differentiation into Cardiomyocytes (Day 5-7): Step 3

1. At the end of this 24 hours (See Step 2, Point 2), gently aspirate Step 2 and 4 media. Introduce room temperature Cardiomyocyte Differentiation Media Step 3. To reconstitute complete CET's Cardiomyocyte Differentiation Media Step 3, thaw CET's Cardiomyocyte Differentiation Media Step 3 Supplement overnight at 4°C (in a refrigerator). **Never thaw supplement by placing it into a 37°C waterbath.** Add the entire contents of the supplement (2 milliliters) to CET's Cardiomyocyte Differentiation Media Step 3. Mix gently. Label, date and place complete Cardiomyocyte Differentiation Media Step 3 in a 4°C refrigerator. This media has a shelf life of 3-weeks if stored at 4°C.

2. Let cells incubate at 37°C for 2 days (48 hours) undisturbed.

Differentiation into Cardiomyocytes (Day 7-9): Step 4

1. At the end of this 48 hours (See Step 3, Point 2), gently aspirate Step 3 media. Introduce room temperature Cardiomyocyte Differentiation Media Step 2 and 4.

2. Let cells incubate at 37°C for 2 days (48 hours) undisturbed.

Differentiation into Cardiomyocytes (Day 9-11): Step 5

1. At the end of this 48 hours (See Step 4, Point 2), gently aspirate Step 2 and 4 media. Introduce room temperature Cardiomyocyte Differentiation Media Step 5. To reconstitute complete CET's Cardiomyocyte Differentiation Media Step 5, thaw CET's Cardiomyocyte Differentiation Media Step 5 Supplement overnight at 4°C (in a refrigerator). **Never thaw supplement by placing it into a 37°C waterbath.** Add the entire contents of the supplement (2 milliliters) to CET's Cardiomyocyte Differentiation Media Step 5. Mix gently. Label, date and place complete Cardiomyocyte Differentiation Media Step 5 in a 4°C refrigerator. This media has a shelf life of 3-weeks if stored at 4°C.

2. Feed cells every 48 hours. Cells will start beating by Day 10-13 depending on the line used and their potential for cardiomyocyte differentiation.

Confirmation of Cardiomyocytes:

1. At the end of 13 days, cells can be screened via immunofluorescence for the expression of Troponin-T and by finding beating cardiomyocyte foci.

All of CET's iPS cell lines are foot-print free, feeder-free and virus-free. All procedures must be done by trained personnel with experience in cell culture. All procedures must be done in a sterile fashion using a laminar flow hood and all cells must be cultured in a 37°C incubator with 95% relative humidity and 5% CO_2. Although it is not necessary, better growth and differentiation are seen in tri-gas incubators capable of reducing Oxygen concentration to 5%.

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Table 1. Preparation of 100 ml complete Induced Pluripotent Stem Cell Cardiomyocytes (CM) Differentiation Media

Brand	Amount for 100 mL	Product	Catalog Number
CET	98 mL	CET CMM Media *Step 1*	CET.DIFF.CMM-100
CET	2 mL	CET Growth Supplement *Step 1*	CET.DIFF.CMM-100
CET	98 mL	CET CMM Media *Step 2 & 4*	CET.DIFF.CMM-100
CET	2 mL	CET Growth Supplement *Step 2& 4*	CET.DIFF.CMM-100
CET	98 mL	CET CMM Media *Step 3*	CET.DIFF.CMM-100
CET	2 mL	CET Growth Supplement *Step 3*	CET.DIFF.CMM-100
CET	98 mL	CET CMM Media *Step 5*	CET.DIFF.CMM-100
CET	2 mL	CET Growth Supplement *Step 5*	CET.DIFF.CMM-100
Any	1 mL for each step	Antibiotic/ Antimycotic solution	Refer to Manufacturer's Catalog Number

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell mark-ers. 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 nega-tive 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.

All products are for research use only. Not for diagnostic or therapeutic use. CET's products are designed and tested to function with other CET products only. For example, all of our cells are optimized to grow and differentiate in CET media. Although investigators are welcome to formulate their own media, CET cannot and will not guarantee that cells will func-tion as indicated in the product brochure. Moreover, such third party use will void CET's obligation to replace cells, should they not function as indicated.

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