SOP for Thawing and Culturing Human iPSC-Derived Cardiomyocytes
Stanford CVI Biobank

The purpose of this document is to outline the standard operating procedures (SOP) for thawing and culturing iPSC derived cardiomyocytes.

From Life Technologies:
B27 supplement minus insulin, Gibco/Life, A1895601
DMEM/F12, Gibco/Life 10565-042

From Fisher Scientific:
RPMI 1640, Fisher/Corning, 10-040-CM
BD Matrigel Basement Membrane Matrix Growth Factor Reduced, Fisher/Corning, 354230

From Sigma:
Accutase, Sigma, A6964-100ml

Protocol:
Note: Each frozen vial of iPSC-CMs should be thawed onto 1 well of a 6-well plate. 48 hours after initial plating, iPSC-CMs can be lifted and re-plated onto any Matrigel-coated Surface.

Note: When re-plating to a smaller surface (i.e. 12, 24, 48, 96-well), calculate the number of cells to re-plate based on the surface area of the new format.
(Surface Area of 6-well / Surface Area of 12-well) = Number of Wells to Split

1. Coat 1 well of a 6 well plate with Matrigel diluted 1:100 with DMEM/F12.
2. Pipet 9 mL of room temperature (RT) RPMI + B-27 + 5% FBS (or DMEM/F12 + 5% FBS) into a 15 mL tube.
3. Thaw a single cardiomyocyte vial in the water bath at 37°C. Hold the vial in the water bath until only a small piece of ice is left.
4. Remove the cardiomyocyte vial from the water bath and spray the vial with 70% EtOH.
5. Transfer the vial to the cell culture hood and pipet the cell suspension drop by drop into the 9 mL of RT media.
6. Centrifuge for 5 min at 200g at RT.

7. Aspirate the supernatant.
8. Suspend the cell pellet in 4 mL of RT RPMI + B-27 + 5% FBS media, pipetting up and down slowly.
9. Aspirate the Matrigel from the coated well of the 6 well dish. Do not let Matrigel coating dry.
10. Add 4 mL of the re-suspended cells to each coated well carefully, distributing them evenly.
11. Place the cells in an incubator at 37°C, 5% CO2, and 85% humidity for 48 hours. Cells should begin to beat 48 hours after plating and can be used for assays from this point.
12. For re-plating to 96 well plates or other surfaces: lift cells using Accutase for 5 minutes at 37°C, then check by light microscopy to confirm CMs have started to detach. If necessary, place iPSC-CMs back at 37°C for another 3-5 minutes.
13. Block the Accutase by adding 10mL of DMEM/F12 + 5-10% FBS to the cell suspension Pipet up and down on surface of plate to further detach CMs.
14. Count cells using a hemocytometer, and re-plate to any Matrigel-coated surface at the desired concentration. Let cells rest at least 48 hours after re-plating before use or until they beat again.
15. Replace media every other day with pre-warmed RPMI+B-27 media.
16. Cells can be used for assays from day 2 - 14 post-thaw (recommended time point is day 5-7).

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