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[MDC_Alternative_2D_hiPSC-CM_diff] hiPSC Cardiomyocyte differentiation V.1

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol outlines a small molecules based, 2D differentiation method for generating high-purity human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Optimized for reproducibility across hiPSC lines, the procedure achieves robust cardiac differentiation through sequential modulation of Wnt signaling, yielding functional beating cardiomyocytes by days 7–10.

Troubleshooting

Day-3 Matrix coating of Culture Vessels

10m

1 Coating of culture vessels (Geltrex, Laminin-511, etc.) according to optimize protocol for hiPSC

10m

Day-3 Seeding hiPSCs

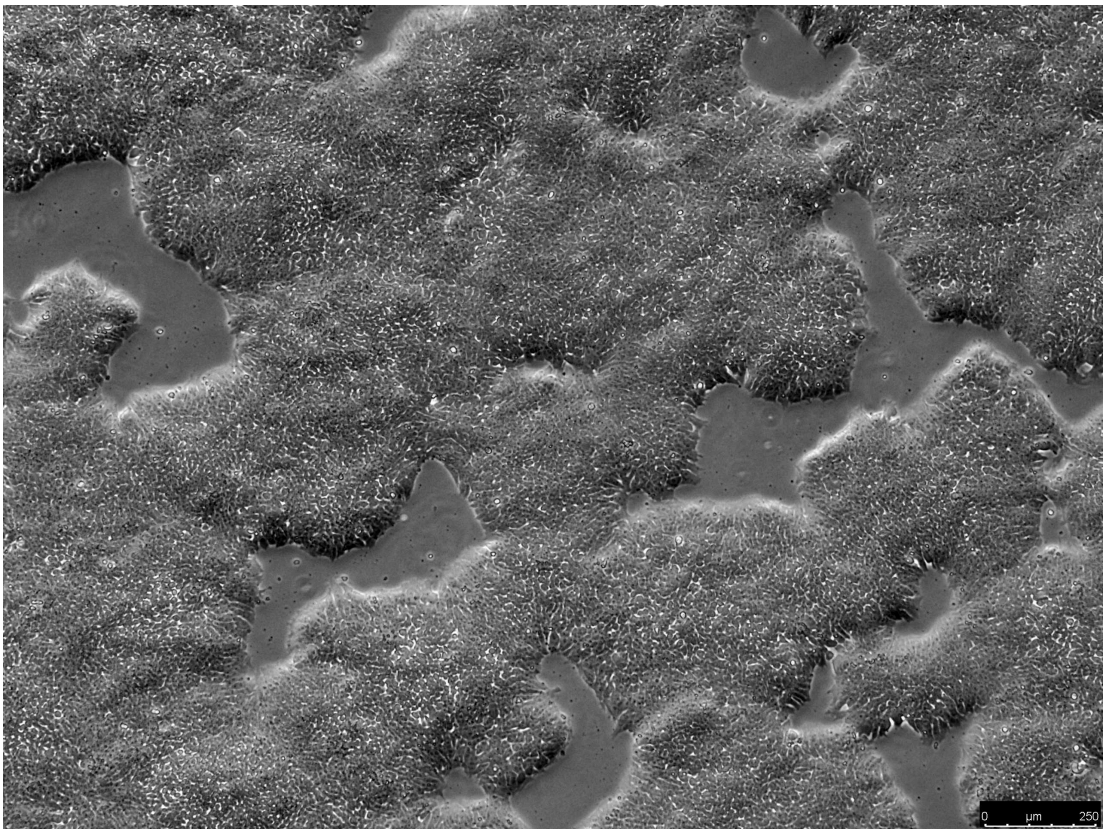
3d

2 Passage hiPSCs as single cells using Accutase or 1x TrypLE after 1x PBS(-) wash

3 Seed hiPSCs on Matrix-coated 6-well plates at a density of 4×10^5 cells/well in E8 medium supplemented with [M] 10 micromolar (µM) ROCK inhibitor for the first 24:00:00 .

4 Incubate in a hypoxia incubator ([M] 5 % CO2 , [M] 5 % O2 , 37 °C).

5 Change the medium to E8 without ROCK inhibitor after 24:00:00 , and continue daily media changes until the cell density reaches [M] 90-95 % (approximately 3-4 days). *Never reach full confluency



hiPSC in E8 (day4) before differentiation, 90% confluency

3d

Day 0-21 Cardiac Differentiation

1w 2d

6 Day 0 (Priming): Change the medium to 2 mL /well of Cardiac: priming medium (RPMI 1640 + B27 Minus Insulin + CHIR-99021). Use a CHIR concentration between [M] 5-10 micromolar (µM) depending on the individual hiPSC line.

7 Day 1: Add 4 mL basal Differentiation medium/well (B27-minus insulin, w/o CHIR-99021) without removing the previous Cardiac: priming medium. 1d

8 Day 3 (Induction): Remove the basal Differentiation medium and add 4 mL of Cardiac: induction medium/well (RPMI 1640 + B27 Minus Insulin + IWR-1-endo). Use [M] 5 micromolar (µM) IWR-1-endo for our iPSC. 2d

9 Day 5: Add 4 mL basal Differentiation medium/well (B27-minus insulin, w/o IWR-1-endo) without removing the previous Cardiac: induction medium.

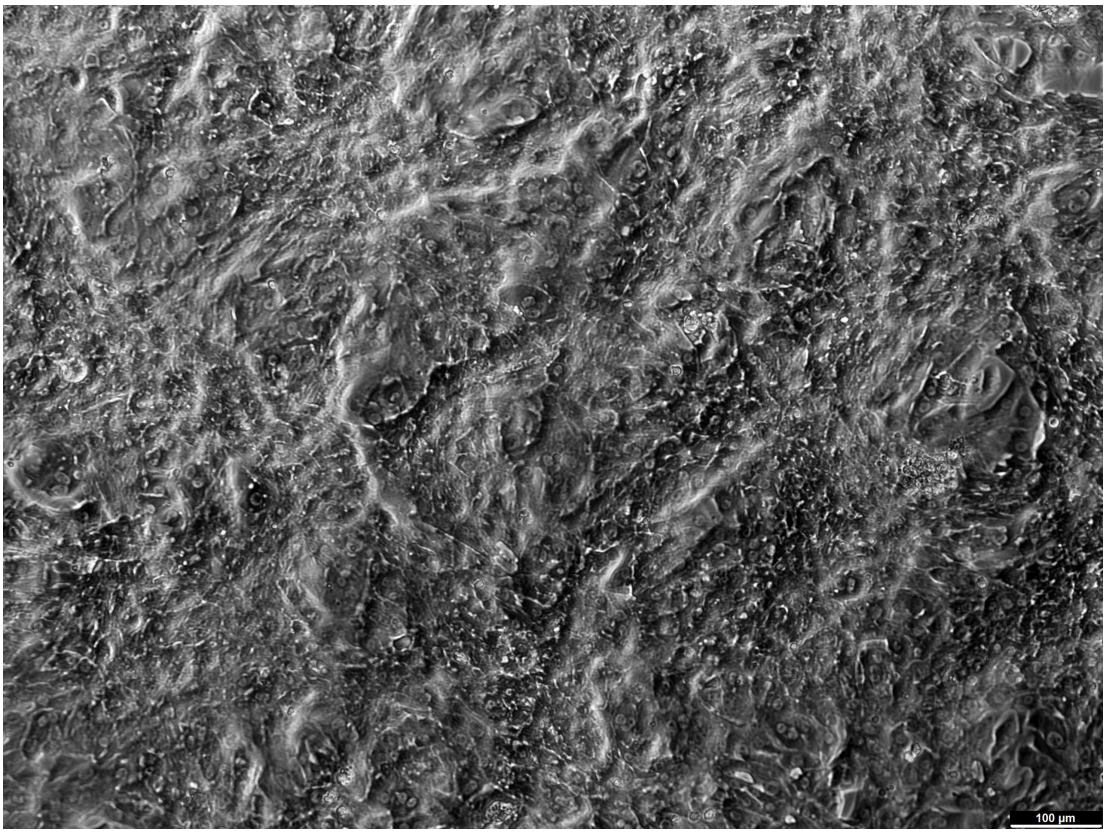
10 Day 7: Remove Differentiation medium and replace with 2-3 mL Maintenance medium (RPMI 1640 + B27)

11 Day 8: Remove Maintenance medium and replace with 2-3 mL Maintenance medium (RPMI 1640 + B27) Beating cells should be observed around days 7-10.

12 Days 9-14: Change Maintenance medium 3 mL every 48:00:00 . 2d

13 Days 9-12 (Optional Lactate Selection): If desired, perform metabolic selection. Replace Maintenance medium with 2 mL Lactate Selection medium/well (RPMI 1640 w/o glucose + CDM3 supplement + Sodium DL-lactate 50 µL). Check for cell death and replace Lactate Selection medium as needed. After 24:00:00 , revert to Maintenance medium if a high proportion of beating hPSC-CMs is observed. 1d

14 Days 15-21: Perform media changes 3-5 mL every 24:00:00 to 48:00:00 with Maintenance medium depends on the color (yellowish: metabolites such as lactate) of medium.



hiPSC-CMs (d20, 1 day Lactate selection) Yield: 146.2 Mio from 18 wells of 6-well plate

3d

Notes

15 Ensure that all steps are performed with sterile techniques.
Optimize CHIR concentration (5-10 µM) for each cell line.
Be gentle during cell resuspension to avoid osmotic shock and shear stress.