Dissociating Cardiomyocytes by Enzymatic Treatment

Introduction

Certain applications require dissociation of an iCell® Cardiomyocytes monolayer into a single cell suspension. Appropriate dissociation of the cardiomyocytes is critical to preserve robust functionality and achieve an adequate cell yield. This Application Protocol details the recommended procedure for dissociating iCell Cardiomyocytes with trypsin.

Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Cardiomyocytes User's Guide.

Item	Vendor	Catalog Number
Equipment		
1 ml Pipettor and Sterile Tips	Multiple Vendors	N/A
Tabletop Centrifuge	Multiple Vendors	N/A
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-010-001 CMC-100-010-005
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14190
Trypsin 0.5%-EDTA (10X), no Phenol Red	Invitrogen	15400

Methods

Appropriately collecting iCell Cardiomyocytes from a cell culture plate as described here is a critical step to achieve robust functionality upon transfer to a secondary plate. iCell Cardiomyocytes are sensitive to over-digestion by the dissociating enzyme and to excessive mechanical trituration. The following recommendations detail proper handling procedures for the dissociating enzyme and iCell Cardiomyocytes cultured for 7 days post-plating in 6-well cell culture plates. Scale volumes appropriately for other cell culture vessel formats.

1. Before use, equilibrate an aliquot of iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) and D-PBS in a 37°C water bath.
2. Dilute 0.5% trypsin solution in D-PBS to a final concentration of 0.1%. Before use, equilibrate the 0.1% trypsin solution in a 37°C water bath.

   Note: *To ensure a consistent dissociation, dispense a stock of 0.5% trypsin solution into single-use aliquots and store frozen until use. Thaw 0.5% trypsin aliquot(s) at 4°C overnight. Always use freshly prepared 0.1% trypsin and equilibrate trypsin in* 37°C *water bath for 10 minutes. Avoid prolonged equilibration periods of time.*
3. Aspirate the Maintenance Medium from the 6-well cell culture plate containing iCell Cardiomyocytes.

   Note: *Dissociate a single 6-well cell culture plate at a time to avoid over-digesting the cardiomyocytes*.
4. Wash the cells twice with 2 ml/well of 37°C D-PBS.
5. Add 1 ml/well of 37°C 0.1% trypsin solution. Incubate in a cell culture incubator at 37°C, 7% CO_2 for 2 minutes.

   Note: *iCell Cardiomyocytes may not appear to be lifting from the bottom of the 6-well cell culture plate at the end of the 2-minute trypsin incubation. Proceed with the next step to wash the cardiomyocytes from the plate to avoid over-digestion with trypsin.*

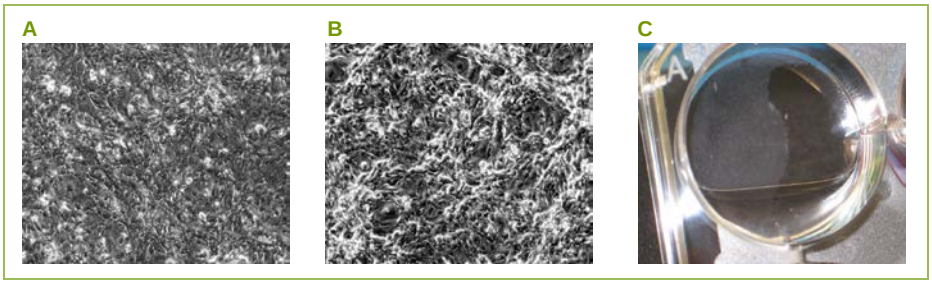

Figure 1: iCell Cardiomyocytes before and after Enzyme Treatment
iCell Cardiomyocytes were cultured for 7 days on a 6-well cell culture plate: (A) confluent monolayer of cardiomyocytes before enzyme treatment, (B) after enzyme treatment, and (C) washed from half of the well as described in step 6.

6. Quickly wash the cardiomyocytes from the plate surface using a 1 ml pipettor by tilting the plate and repeatedly aspirating and dispensing the trypsin solution over the plate surface 2 - 3 times (Figure 1C).
7. Rotate the plate 180° and repeat the previous step.
8. Add 3 ml/well of 37°C Maintenance Medium to quench the trypsin.
9. Tilt the plate at a 45° angle and triturate the cells 3 - 4 times using a 5 ml serological pipette to individualize the cells. Avoid over trituration and introduction of air bubbles.

   Note: *Triturate the cells from each well before transferring into the conical tube. Cardiomyocytes might reattach to the bottom of the plate if not individualized quickly.*

Notes

Notes

10. Transfer the iCell Cardiomyocytes cell suspension to a 50 ml conical tube.

    Note: *Use a 1 ml pipettor to collect any remaining cardiomyocyte suspension from the wells after the cell transfer.*

11. Centrifuge the cell suspension at room temperature, 180 x g for 5 minutes.

12. Aspirate the supernatant, being careful not to disturb the cell pellet. Gently resuspend the cell pellet in 3 ml of Maintenance Medium per 6-well cell culture plate of dissociated cardiomyocytes using a 5 ml serological pipette.

    Note: *CDI recommends to resuspend the cells at a high density to avoid additional centrifugation steps to achieve the appropriate cell concentration.*

13. Remove an aliquot to count the fraction of viable cardiomyocytes using a hemocytometer.

    Note: *Ensure the cardiomyocytes are suspended evenly before removing an aliquot to count.*

14. Dilute the cell suspension in Maintenance Medium to the appropriate cell concentration to achieve the desired seeding density.

    Note: *CDI recommends waiting at least 2 - 3 days following dissociation to assess functionality.*

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