Dissociating Hepatocytes by Enzymatic Treatment

Introduction

Certain applications require dissociation of an iCell® Hepatocytes 2.0 monolayer for transfer to a 2D plate or 3D culture system. Appropriate dissociation of the hepatocytes is critical to preserve robust functionality and achieve an adequate cell yield. This Application Protocol details the recommended procedure for dissociating iCell Hepatocytes 2.0 by enzymatic treatment using a dissociation reagent.

Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Hepatocytes 2.0 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 Hepatocytes 2.0 Kit (Hepatocytes)	Cellular Dynamics International (CDI)	PHC-100-020-001
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	ThermoFisher Scientific	14190
Dissociation Reagent*	ThermoFisher Scientific	

* The cell culturing procedures described here were optimized using either TrypLE Express Enzyme (ThermoFisher Scientific, Cat. No. 12563) or StemPro Accutase Cell Dissociation Reagent (ThermoFisher Scientific, Cat. No. A11105).

Methods

The following procedure details how to prepare the hepatocytes cultured in 6-well cell culture plates for dissociation and transfer to a 2D plate or 3D culture system. Scale volumes appropriately for other well formats.

Culturing Hepatocytes

Thaw and maintain the hepatocytes according to their User's Guide until ready to perform dissociation.

Collecting Hepatocytes

Notes

Appropriately collecting hepatocytes from a cell culture plate as described here is a critical step to preserve robust functionality upon transfer to a 2D plate or 3D culture system. The hepatocytes are sensitive to over-digestion by the dissociation reagent and to excessive mechanical trituration. The following recommendations detail proper handling procedures for the dissociation reagent and hepatocytes cultured for 7 days post-plating in 6-well cell culture plates. Scale volumes appropriately for other well formats. Before use, equilibrate an aliquot of iCell Hepatocytes Maintenance Medium (Maintenance Medium) and D-PBS to room temperature. It is not necessary to equilibrate the dissociation reagent.

1. Remove the 6-well cell culture plate containing hepatocytes from the incubator.
2. Aspirate the spent medium.

   Note: *Dissociate a single 6-well cell culture plate at a time to avoid over-digesting hepatocytes.*
3. Wash the cells twice with 2 ml/well of room temperature D-PBS.
4. Add 1 ml/well of dissociation reagent.
5. Incubate at room temperature until cells just begin to detach from the plate (approximately 2 - 4 minutes).

   Note: *Observe cells under a microscope to determine when detachment occurs.*
6. Add 2 ml/well of Maintenance Medium to dilute the dissociation reagent.
7. Gently pipette up and down several times around the well to ensure the cells are detached and to obtain a cell suspension.

   Note: *The cell suspension contains mostly small clusters of cells. Do not attempt to singularize the cells. Over-digestion of the hepatocytes impairs their functionality in a 2D plate or 3D culture system.*

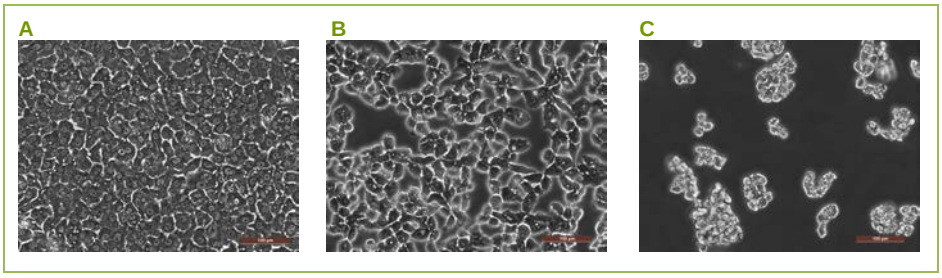

Figure 1: Hepatocytes before and after Enzymatic Treatment Using the Dissociation Reagent
iCell Hepatocytes 2.0 were cultured for 7 days on a 6-well cell culture plate: (A) before treatment, (B) after treatment, and (C) after being washed from the well as described in step 7.

8. Transfer the cell suspension to a 15 ml centrifuge tube.
9. Rinse the well with 3 ml/well of Maintenance Medium and add this volume to the 15 ml centrifuge tube.
10. Centrifuge the cell suspension at 200 x g at room temperature for 5 minutes.

Notes

11. Aspirate the supernatant, being careful not to disturb the cell pellet.
12. Gently resuspend the cell pellet in Maintenance Medium to the desired final cell concentration for transfer to a 2D plate or 3D culture system.

    Note: *Because the cell suspension contains mostly small clusters of cells, it is not amenable to counting. The number of cells recovered from a 6-well cell culture plate seeded with 300,000 cells/cm^2 is estimated to be approximately 1 million cells/well.*

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