StemBioSys®

iCell® Cardiomyocytes² Application Protocol

Plating iCell® Cardiomyocytes² on CELLvo™ MATRIX PLUS

Introduction

iCell® Cardiomyocytes² are cardiac myocytes derived from human induced pluripotent stem cells (iPSC). These cardiomyocytes, which are an extension of the well-characterized iCell product line, have been optimized for faster recovery from cryopreservation and can be used in many functional applications.

The recent article by Block et al. in *Scientific Reports* indicates that iCell Cardiomyocytes² have enhanced functional and structural benefits following culture on CELLvo™ Matrix Plus plates for 7 days. This protocol was developed by Fujifilm Cellular Dynamics, Inc. in collaboration with StemBioSys, Inc., and details the steps for plating iCell Cardiomyocytes² on the CELLvo™ Matrix Plus plates.

Block, T. et al. (2020) *Scientific Reports* 10, Article number: 19071.

Required Equipment, Consumables, and Software

The following equipment and consumables are required in addition to the materials specified in the iCell Cardiomyocytes² User's Guide.

Item/Equipment	Vendor(s)	Catalog Numbers
Multichannel pipettor, 8 or 12 channels	Multiple Vendors	
Consumables		
CELLvo™ Matrix Plus	StemBioSys, Inc.	
• CELLvo™ Matrix Plus 6-well plates	StemBioSys, Inc.	AF-HPME-6WP
• CELLvo™ Matrix Plus 96-well plates	StemBioSys, Inc.	AF-HPME-96WP
iCell Cardiomyocytes² Kit, 01434 or 11713	FUJIFILM Cellular Dynamics, Inc. (FCDI)	R1017, R1218, or R1219
• iCell Cardiomyocytes Plating Medium, 30 ml	FUJIFILM Cellular Dynamics, Inc. (FCDI)	(included in kit)
• iCell Cardiomyocytes Maintenance Medium, 100 ml	FUJIFILM Cellular Dynamics, Inc. (FCDI)	(included in kit)
Hank's Balanced Salt Solution (HBSS) Ca2+ and Mg2+	Millipore Sigma	55037C
Pipette Tips	Multiple Vendors	

Tips Before Starting

1. Upon receipt, store CELLvo Matrix Plus plates at 4°C.
2. Refer to the iCell Cardiomyocytes² User's Guide for information on storage and handling of the cells and media.
3. Read this entire Application Protocol before handling iCell Cardiomyocytes² to become familiar with assay workflow.
4. Thaw both bottles of media required for this assay, including iCell Cardiomyocytes Plating Medium (Plating Medium, 30 ml) and iCell Cardiomyocytes Maintenance Medium (Maintenance Medium, 100 ml), overnight at 4°C the day prior to thawing and plating cells.

Methods

CELLvo Matrix Plus Plate Preparation

1. Transfer CELLvo Matrix Plus plates to room temperature on the day of cell plating.
2. To reconstitute the CELLvo Matrix Plus plates, add Hank's Balanced Salt Solution (HBSS; 1 ml per well of a 6-well plate or 50 µl per well of a 96-well plate) and incubate the plate for 1 hour in a 37°C, 5% CO_2 incubator.
3. Aspirate the HBSS from each well and wash 2 times with HBSS.
4. Aspirate the HBSS immediately before seeding the iCell Cardiomyocytes2..

Thawing iCell Cardiomyocytes^2

1. Warm Plating Medium to 37°C in a water bath prior to use.
2. Thaw cells into a sterile 50 ml centrifuge tube according to the iCell Cardiomyocytes2 User's Guide.

   ***Note**: The total volume of cell suspension at thaw is 5 ml (1 ml cryovial contents + 1 ml Plating Medium rinse + 3 ml of additional Plating Medium), which is less than the amount listed in Chapter 5 of the User's Guide.*
3. To confirm cell viability and count, remove a sample of cells to be examined in a hemocytometer with trypan blue or an automated cell counter.
4. Calculate the final volume of Plating Medium needed to obtain a final cell plating density of 1 x 10^6 viable cardiomyocytes/ml using the number of viable cells/vial from the Certificate of Analysis (COA).

   ***Note**: Each Certificate of Analysis can be found online here: fujifilmcdi.com/resources/coa-lookup/*

Plating iCell Cardiomyocytes^2 onto the CELLvo Matrix Plus Plate

1. Adjust the volume with Plating Medium to obtain the desired concentration of cells (see recommended cell densities for each plate size below). CELLvo Matrix Plus is suitable for single cell or functional syncytia preparations.
   - For a 96-well plate, functional syncytia of iCell Cardiomyocytes^2 may be obtained by seeding 75,000 cells/well.
   - For a 6-well plate, functional syncytia of iCell Cardiomyocytes^2 may be obtained by seeding 1,500,000 cells/well.
2. Add the adjusted cell suspension to each well of the plate.
3. Incubate the cells in a cell incubator at 37°C, 5% CO_2 for 4-24 hours.

Culturing of iCell Cardiomyocytes2 in the CELLvo Matrix Plus Plate

1. Equilibrate Maintenance Medium to 37°C in a water bath prior to use.
2. Replace 100% of the medium with 37°C Maintenance Medium (96-well plate: 100 µl/well; 6-well plate: 3 ml/well).
3. Maintain the cardiomyocytes in the CELLvo Matrix Plus plate, replacing 100% of the spent medium with Maintenance Medium every 2 days.
4. Culture cells in a cell incubator at 37°C, 5% CO_2 for additional 5 days before performing experiments. iCell Cardiomyocytes^2 are ready for experiments at Day 7 of culture.

   ***Note**: iCell Cardiomyocytes^2 may be cultured on CELLvo™ Matrix Plus for periods longer than 7 days.*

Representative Data

iCell Cardiomyocytes^2 exhibited differences in morphology when plated on fibronectin verses CELLvo™ Matrix Plus. When the cells were plated on a fibronectin, the iCell Cardiomyocytes^2 showed a circular morphology without alignment (A and C), while when they were plated on CELLvo™ Matrix Plus, the iCell Cardiomyocytes^2 showed a rod-shaped morphology with aligned cells (B and D).

A. Fibronectin Matrix **B. CELLvo™ Matrix Plus**

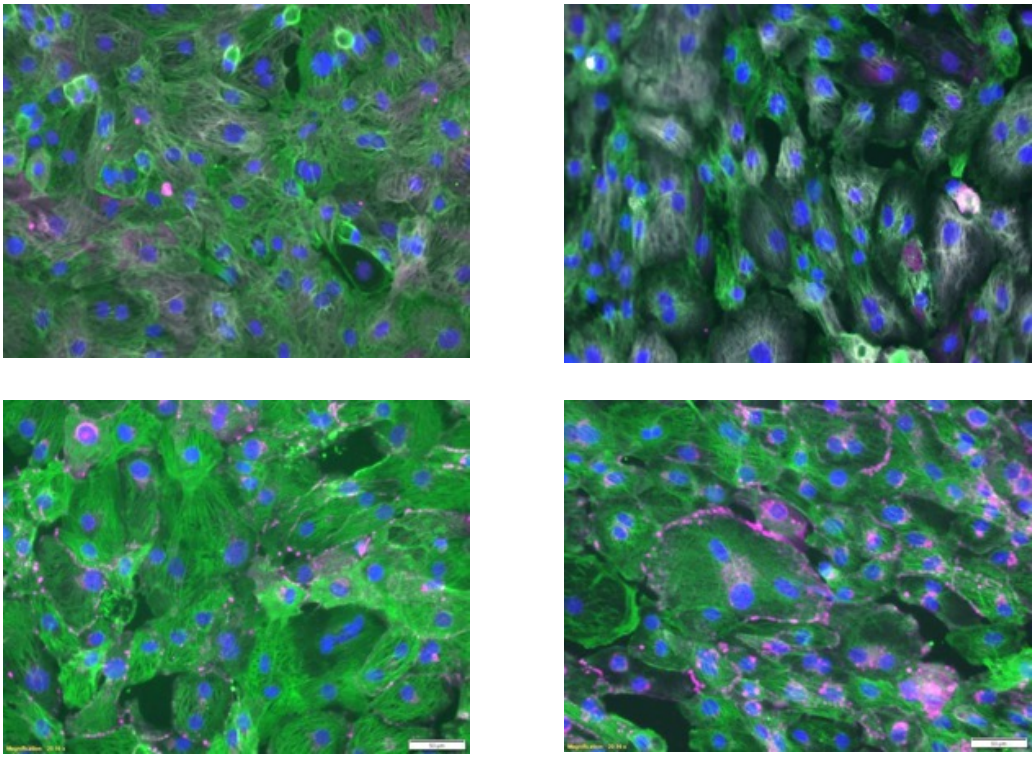

Figure 1. The Effects of Matrices on the Morphology of iCell Cardiomyocyte^2.

These images show iCell Cardiomyocytes labeled for these proteins on various matrices: (A and B) cardiac troponin T (CTNT; green) and cardiac troponin I (CTNI; pink); and (C and D) cardiac troponin T (CTNT; green) and connexin 43 (CX43; pink). Nuclei were stained with Hoechst

CELLvo™ Matrix Plus Maybe covered in part or in whole by US Patent #'s 16/797,945, 16/592,539

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