

# **Modeling Cardiac Proliferation: *bFGF Induction with High Content Analysis***

## **Introduction**

The ability of cardiac progenitor cells to proliferate and differentiate into cardiomyocytes is fundamental during cardiac development in the embryonic and postnatal heart and contributes to the myocyte replacement in a damaged adult heart. iCell® Cardiac Progenitor Cells are human induced pluripotent stem cell-derived cardiac progenitor cells that recapitulate the physiological characteristics of native human cardiac progenitor cells. Due to their human origin, high purity, functional relevance, and ease of use, iCell Cardiac Progenitor Cells represent an optimal in vitro test system for interrogating cardiac regenerative biology in basic research and many areas of drug development. The protocol presented here has demonstrated utility in inducing proliferation of iCell Cardiac Progenitor Cells with basic fibroblast growth factor (bFGF), assessed by the expression of NKX2.5 using high content analysis.

## **Required Equipment and Consumables**

The following equipment and consumables are required in addition to the materials specified in the iCell Cardiac Progenitors Cells Prototype User's Guide.

Item	Vendor	Catalog Number
<b>Equipment</b>		
High Content Imaging System	Multiple Vendors	
<b>Consumables</b>		
bFGF from Zebrafish	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca <sup>2+</sup> and Mg <sup>2+</sup> (D-PBS)	Multiple Vendors	
Formaldehyde, 37%	Multiple Vendors	
Hoechst 33342, 10 mg/ml	Life Technologies	H3570
Nonfat Dry Milk	Multiple Vendors	
Triton X-100	Sigma	93443

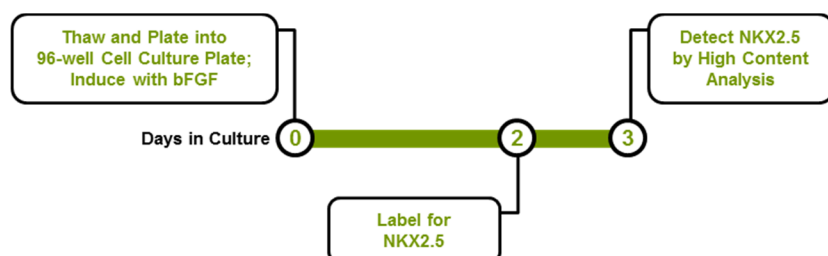
## **Recommended Antibodies**

The following table of primary and secondary antibodies provides the dilution factor to use for labeling iCell Cardiac Progenitor Cells. Select the appropriate combination of primary and secondary antibodies.

Item	Vendor	Catalog Number	Dilution Factor
<b>Primary Antibodies</b>			
Mouse Anti-cardiac Troponin T	Thermo Fisher	MS-295-P	1:100
Rabbit Anti-NKX2.5	Abcam	Ab35842	1:100
<b>Secondary Antibodies</b>			
Cy5 Goat Anti-mouse IgG	Life Technologies	A10524	1:200
Alexa Fluor 488 Donkey Anti-rabbit IgG	Life Technologies	A21206	1:200

## Workflow

iCell Cardiac Progenitor Cells are thawed and plated in bFGF-containing Maintenance Medium (Maintenance Medium) into a 96-well cell culture plate previously coated with fibronectin. On day 2 post-plating, cells are labeled with primary antibodies. On day 3 post-plating, cells are labeled with secondary antibodies and NKX2.5 expression detected.



## Methods

### Thawing iCell Cardiac Progenitor Cells

1. Thaw iCell Cardiac Progenitor Cells according to the iCell Cardiac Progenitor Cells Prototype User's Guide.
2. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
3. Dilute the cell suspension in Maintenance Medium to  $2.8 \times 10^5$  viable cells/ml.

### Plating iCell Cardiac Progenitor Cells

The following procedure details plating iCell Cardiac Progenitor Cells in a 96-well cell culture plate. Scale volumes appropriately for other vessel formats.

1. Reconstitute bFGF in sterile water at 1 mg/ml according to the manufacturer's instructions.

**Note:** If necessary, aliquot and store reconstituted bFGF at  $-20^{\circ}\text{C}$ .

2. Dilute 1 mg/ml bFGF to a final concentration of  $1 \mu\text{g/ml}$  in Maintenance Medium. See the iCell Cardiac Progenitor Cells Prototype User's Guide for medium preparation.
3. Aspirate the fibronectin solution from a pre-coated 96-well cell culture plate.

4. Invert the cell suspension 6 times. Immediately dispense 90  $\mu$ l/well of cell suspension (~25,000 viable cells/well).
5. Immediately add 10  $\mu$ l of 1  $\mu$ g/ml bFGF into experimental wells containing 90  $\mu$ l/well cell suspension. Mix gently by pipetting. Do not add bFGF in control wells.
6. Culture iCell Cardiac Progenitor Cells in a cell culture incubator at 37°C, 7% CO<sub>2</sub> for 48 hours.

#### **Labeling of iCell Cardiac Progenitor Cells: Fixation, Permeabilization, and Primary Antibody Incubation**

The following procedure details labeling iCell Cardiac Progenitor Cells cultured in a 96-well cell culture plate. Scale volumes appropriately for other vessel formats.

1. Prepare 10 ml of fixative solution by adding 1.08 ml of 37% formaldehyde in 8.92 ml of D-PBS to achieve a final concentration of 4% formaldehyde (v/v).
2. Aspirate or quickly decant the spent medium. Wash the cells with 100  $\mu$ l/well of D-PBS.
3. Incubate the cells with 100  $\mu$ l/well of fixative solution at room temperature for 15 minutes.
4. Prepare 50 ml permeabilization buffer by combining 1.5 g of nonfat dry milk (3% w/v) and 50  $\mu$ l of Triton X-100 (0.1% v/v) in D-PBS. Mix by stirring or inverting.
5. Aspirate or quickly decant the fixative solution. Wash 2 times with 200  $\mu$ l/well of D-PBS.
6. Incubate the cells with 100  $\mu$ l/well of permeabilization buffer at room temperature for 15 minutes, protected from light.
7. Prepare the primary antibody solution by diluting the primary antibodies in permeabilization buffer to 1:100.
8. Aspirate or quickly decant the permeabilization buffer.
9. Incubate the cells with 50  $\mu$ l/well of primary antibody solution at 4°C overnight, protected from light.

#### **Labeling iCell Cardiac Progenitor Cells: Secondary Antibody Incubation and Nuclei Staining**

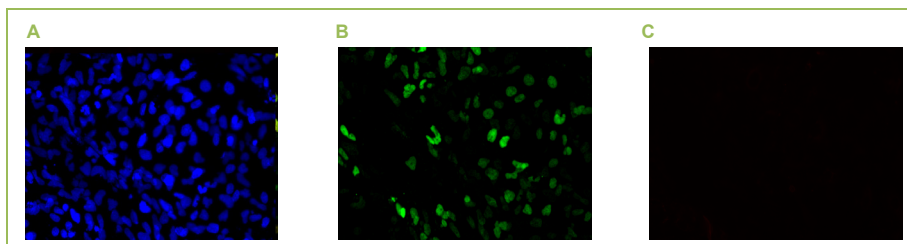
1. Prepare the secondary antibody solution by diluting the secondary antibodies in D-PBS to 1:200.
2. Aspirate or quickly decant the primary antibody solution. Wash 3 times with 200  $\mu$ l/well of D-PBS.
3. Incubate the cells with 50  $\mu$ l/well of secondary antibody solution at 4°C for 1 hour, protected from light.
4. Prepare the nuclei staining solution by diluting the Hoechst 33342 in D-PBS to 1:5,000.
5. Add 50  $\mu$ l/well of nuclei staining solution in the 96-well cell culture plate already containing 50  $\mu$ l/well of secondary antibody solution. Incubate the cells at room temperature for 10 minutes, protected from light.

- Aspirate or quickly decant the secondary antibody and nuclei staining solution. Wash 2 times with 200  $\mu$ l/well of D-PBS.
- Add 100  $\mu$ l/well of D-PBS and prepare for high content analysis.

### Data Analysis

See the guide for the high content imaging system for data analysis instructions.

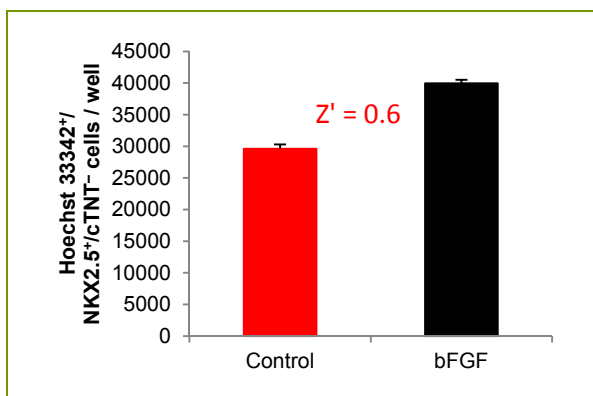
- Use the area of Hoechst 33342 signal (blue), NKX2.5 signal (green), and cTNT signal (red) to define the iCell Cardiac Progenitor Cells as a Hoechst 33342<sup>+</sup>/NKX2.5<sup>+</sup>/cTNT<sup>-</sup> cell population (Figure 1).



**Figure 1: Definition of the iCell Cardiac Progenitor Cells Population by High Content Analysis**

These images show iCell Cardiac Progenitor Cells as a Hoechst 33342<sup>+</sup>/NKX2.5<sup>+</sup>/cTNT<sup>-</sup> cell population. iCell Cardiac Progenitor Cells were cultured in the presence of bFGF for 2 days. Nuclei were stained with Hoechst 33342 (blue, panel A). Cells were labeled with antibodies to detect NKX2.5 (green, panel B) and cTNT (red, panel C).

- Plot the number of Hoechst 33342<sup>+</sup>/NKX2.5<sup>+</sup>/cTNT<sup>-</sup> cells (Y-axis) against the experimental condition (X-axis) (Figure 2).




**Figure 2: Detection and Quantification of bFGF-induced Cardiac Proliferation by High Content Analysis**

In this representative experiment, cell proliferation of iCell Cardiac Progenitor Cells was induced as indicated by the increase in the Hoechst 33342<sup>+</sup>/NKX2.5<sup>+</sup>/cTNT<sup>-</sup> cell population in the treatment condition. Acquisition and analysis were performed using the ImageXpress Micro System and MetaXpress Software. iCell Cardiac Progenitor Cells were plated in the presence of bFGF for 2 days and assayed on day 2 post-plating.

## Summary

iCell Cardiac Progenitor Cells are derived from human iPSCs and provide an in vitro cellular system for modeling cardiac regeneration. The methods and data presented here highlight a reproducible cellular assay for assessing bFGF-induced cardiac proliferation by monitoring and quantifying the Hoechst 33342<sup>+</sup>/NKX2.5<sup>+</sup>/cTNT<sup>-</sup> cardiac progenitor cell population by high content analysis.

© 2014 Cellular Dynamics International, Inc. All rights reserved.  
For Life Science Research Use Only.

iCell is a registered trademark, and Cellular Dynamics, the  logo, and MyCell are trademarks of Cellular Dynamics International, Inc.

All other brands, product names, company names, trademarks, and service marks are the properties of their respective owners.

Version: December 2014  
AP-CPCPRO141201