

iCell® Cardiac Products:

Applying Transfection Technologies to Create Novel Screening Models

Cellular Dynamics International has developed and commercialized a wide range of human iPS cell-derived cell types including iCell® Cardiomyocytes and iCell Cardiomyocytes². These cells have demonstrated biological relevance and functional utility in various drug screening and toxicity applications.¹ Combining these attributes with molecular techniques to modulate endogenous genetic elements or introduce new elements and markers expands the utility of iPSC-derived models.

This Application Note highlights RNAi and plasmid DNA transfection techniques for target gene/pathway modulation and reporting in iCell Cardiomyocytes and iCell Cardiomyocytes² and highlights three advantages of human iPS cell-based systems over traditional primary cell models: (1) high efficiency/low toxicity transfection, (2) flexible and prolonged expression windows, and (3) real-time, multiplexed functional readouts.

Methods

Plasmid DNA Transfection. iCell Cardiomyocytes and iCell Cardiomyocytes² (Cellular Dynamics, #CMC-100-010-001, #CMC-100-012-001, respectively) were plated in 96-well cell culture plates according to the User's Guide. Plasmid DNA encoding green fluorescent protein (pZsGreen1-N1, Clontech, #632448) was delivered into the cells according to the manufacturer's instructions for each transfection reagent.^{2,3} The transfection efficiency was assessed by fluorescence microscopy or flow cytometry (represented as a percentage of GFP-expressing cells). Optimal cell culture, transfection, and analysis parameters are provided in Figure 1.

siRNA Transfection. iCell Cardiomyocytes were transfected with siRNA oligonucleotides to knock down GAPDH.⁴ iCell Cardiomyocytes were plated in 12-well cell culture plates and cultured for 7 days according to

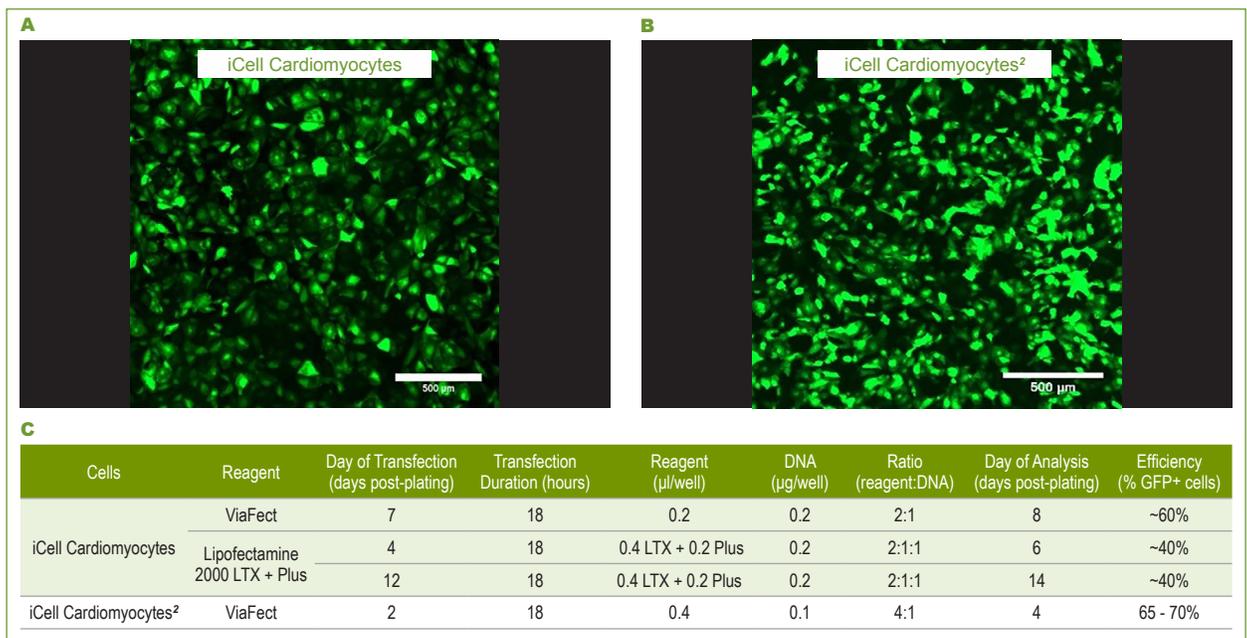
the User's Guide. TransIT-TKO Transfection Reagent (Mirus Bio, #MIR 2150) was used to form complexes with the siRNA oligonucleotide at a ratio of 4 µl:50 nM per well. Then, the cells were incubated with the transfection complex, and the effect of RNAi-mediated knockdown of GAPDH was measured 72 hours post-transfection. The expression levels of GAPDH (relative to 18s RNA) in test samples were normalized to controls (cells transfected with a scrambled siRNA oligonucleotide sequence) (Figure 2).

Reporter Construct Transfection. Activation of the cAMP response element (CRE) was measured by transiently transfecting iCell Cardiomyocytes with 0.2 µg/well of a reporter gene construct carrying a CRE-responsive luciferase (pGL4.29, Promega, #E8471) using 0.4 µl/well of Lipofectamine 2000 LTX and 0.2 µl/well of Plus Reagent (Life Technologies, #15338). On day 1 or day 9 post-transfection, cells were incubated with 3-fold dilution doses of isoproterenol and luciferase activity was measured 24 hours post-treatment (Figure 3). Multiplex analysis of stimulation of intracellular cAMP pathway was measured by transiently co-transfecting iCell Cardiomyocytes on day 7 post-plating with 0.5 µg/well of a CRE-luciferase reporter system (pNL[NlucP/CRE/Hygro] Vector, Promega, contact their Custom Assay Services for catalog #) and 0.5 µg/well of an intracellular cAMP biosensor (pGloSensor-22F cAMP Plasmid, Promega, #E230A). On day 1 post-transfection, cells were incubated with the adenylate cyclase activator forskolin, and the biosensor activity was assessed in real-time/kinetic mode immediately after forskolin addition using the GloSensor cAMP Assay (Promega, #E1290) (Figure 4A). In the same cells, the CRE transcription response was measured 4 hours post-treatment using the Nano-Glo Luciferase Assay (Promega, #N1130) (Figure 4B).

Results & Discussion

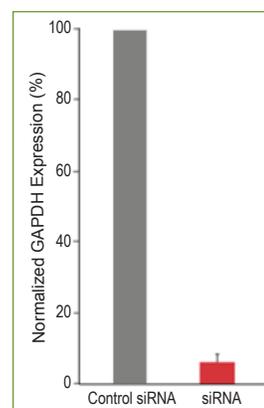
Methods commonly used to transfect cell lines often fail when applied to primary cells. Thus, biological pathways are often examined in artificial systems, such as heterologous cell lines. iPSC-derived cells overcome these hurdles by recapitulating typical human biology while remaining amenable to transfection methods. Transfection of iCell Cardiomyocytes and iCell Cardiomyocytes² was evaluated with plasmid DNA using commercially available lipid-based transfection reagents

(Figure 1). The transfection conditions were optimized with different amounts of DNA and transfection reagent according to individual manufacturer's instructions. iCell Cardiomyocytes and iCell Cardiomyocytes² were tested on several days post-plating, providing transfection conditions for short- and long-term cultures (Figures 1 and 3). Using optimized conditions (Figure 1C), high transfection efficiencies and low cytotoxicities were achieved.

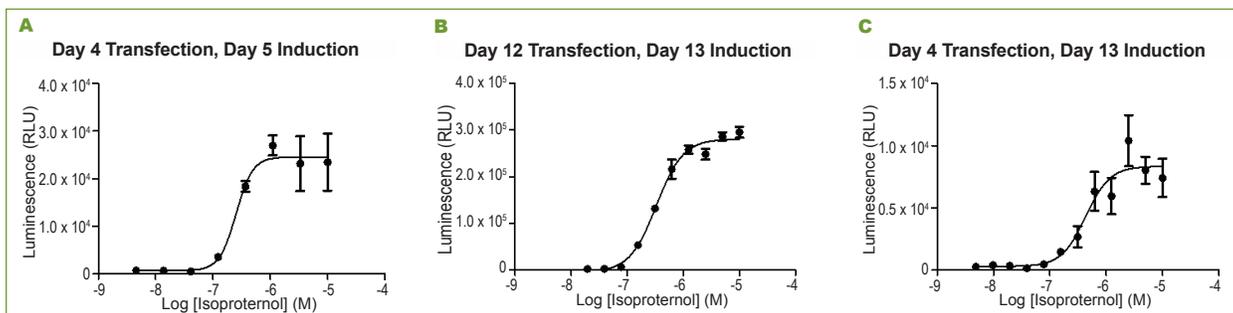


▲ **Figure 1: Commercially Available Transfection Techniques Produce High Transfection Efficiencies in iCell Cardiac Products** iCell Cardiomyocytes and iCell Cardiomyocytes² overcome common limitations in transfecting primary cardiomyocytes by supplying a human-based system for nucleic acid delivery with high efficiency and minimal toxicity. (A, B) Fluorescent microscopy shows GFP expression in cardiomyocytes transfected with ViaFect Transfection Reagent on the day of analysis (40X total magnification). (C) The table lists comparative data of transfection conditions.

The delivery of nucleic acids to cultured cells can be employed in gene expression modulation studies directed towards elucidating or interfering with a cellular mechanism. Exogenously applied siRNAs offer a simple method to knock down the expression of target genes. Efficient RNA interference in iCell Cardiomyocytes was demonstrated using a transiently transfected siRNA oligonucleotide designed to silence GAPDH, a common housekeeping gene (Figure 2). Optimized culture and reagent conditions enabled siRNA delivery in iCell Cardiomyocytes as demonstrated by the effective knockdown of target gene expression.



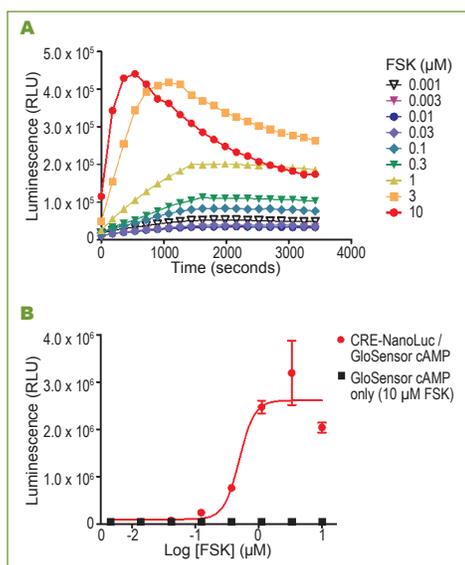
◀ **Figure 2: iCell Cardiac Products Provide a Relevant System for the Knockdown of Endogenous Genes** The expression of genes and proteins at endogenous levels in iCell Cardiomyocytes compared to recombinant cell lines enables target identification and validation studies in a clinically relevant system. iCell Cardiomyocytes exhibit a reduction of GAPDH mRNA levels following transfection using target-specific siRNA compared to the control siRNA (mean ± SEM, n = 3).



▲ Figure 3: Transient Transfections of iCell Cardiac Products Can Provide Long-term Readouts
 Unlike most heterologous cell systems where transient transfections are temporary, transfection into iCell cardiac products provide an extended window for analysis. iCell Cardiomyocytes were transfected transiently with a CRE-responsive luciferase reporter construct on (A, C) day 4 and (B) day 12 post-plating. The β -adrenergic pathway was stimulated with isoproterenol on (A, B) day 1 and (C) day 9 post-transfection, and luciferase activity was analyzed 24 hours post-treatment (mean \pm SEM, n = 3). EC₅₀ values for isoproterenol determined in the representative experiments were (A) 0.2 μ M, (B) 0.3 μ M, and (C) 0.4 μ M.

Using reporter gene constructs and a relevant cellular model is particularly important when interrogating endogenous signaling pathways. iCell cardiac products offer an optimal test system for screening biochemical pathways by combining biological relevance and easy delivery of reporter gene constructs. iCell Cardiomyocytes were transiently transfected with CRE-responsive luciferase reporter constructs that allow for monitoring the β -adrenergic receptor signal transduction pathway. In Figure 3, the luciferase activity was analyzed at two time points after transfection to investigate changes in pathway activation over time. Cells were stimulated with isoproterenol and

dose-response curves were generated. In Figure 4, the cAMP levels were stimulated with the adenylate cyclase activator forskolin. The activity was analyzed using multiplex reporter assays consisting of a cAMP biosensor to monitor in real time the kinetics of cAMP pathway activation and of a CRE-reporter to measure transcriptional activation downstream of the pathway stimulation. These data illustrate that the CRE-luciferase constructs were transfected efficiently, the endogenous signaling pathway was induced at protein stimulation as well as transcriptional levels, and the signaling activity was maintained over time in culture.



◀ Figure 4: Pharmacological Responses Can Be Assessed in iCell Cardiac Products Using a Multiplexed Reporter Readout
 The functional relevance of iCell cardiac products enables mechanistic studies on endogenous pathways. iCell Cardiomyocytes were transfected transiently with reporter vectors and stimulated at 24 hours post-transfection with the adenylate cyclase activator forskolin (FSK). (A) Kinetics of increasing intracellular cAMP levels in response to increasing concentrations of forskolin measured in real time by the cAMP biosensor. (B) Forskolin-dependent stimulation of intracellular cAMP pathway analyzed using a CRE-luciferase reporter system multiplexed with the GloSensor cAMP Assay after a 4 hour pathway stimulation with forskolin.

Conclusion

iCell cardiac products can be transfected efficiently with limited cytotoxicity using commercially available transfection reagents. This distinct advantage of human iPSC-derived cardiomyocytes over primary cells, combined with the functional properties of iCell Cardiomyocytes and iCell Cardiomyocytes², offers an easy-to-use cellular system to perform exogenous gene delivery, endogenous gene modulation, and induction of specific cellular responses.⁵ The ease of handling of iCell cardiac products and the stability over time of the cellular functionality in culture allow for both short- and long-term studies with transient transfection. These capabilities enable a wide range of academic and pharmaceutical research applications, in particular high throughput screening and drug discovery.

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