Cellular Dynamics international

Application Note

iCell® Products:

Applying Transfection Technologies to Create Novel Screening Models

The availability of unlimited quantities of high quality, high purity human induced pluripotent stem cell (iPSC)-derived cells offers new opportunities for the generation of novel, physiologically relevant cellular models for drug screening and development. Realizing the full potential of these cellular models could require molecular techniques that enable introduction of exogenous genes and/or modulation of endogenous genetic elements.

Cellular Dynamics International has developed and commercialized human iPS cell-derived iCell® Cardiomyocytes, iCell Neurons, iCell Endothelial Cells and iCell Hepatocytes. These cells have demonstrated biological relevance and functional utility in various drug screening and toxicity applications (1).

This Application Note highlights how iCell products are compatible with a variety of commercially available transfection reagents, thus enabling the regulation of specific target genes for numerous applications in drug discovery research. Transfection reagents were evaluated for delivery of plasmid DNA, small interfering RNA (siRNA) oligonucleotides, and reporter gene constructs.

Methods

Plasmid DNA Transfection. iCell Cardiomyocytes, iCell Neurons, and iCell Endothelial Cells (Cellular Dynamics, #CMC-100-010-001, #NRC-100-010-001, #ECC-100-010-001, respectively) were transfected using reagents from multiple manufacturers. iCell products were plated in 96-well plates according to the relevant 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. The transfection efficiency was assessed by fluorescence microscopy and measured by flow cytometry (represented as a percentage of GFP-expressing cells). Optimal cell culture, transfection, and analysis parameters for each iCell product are provided in Figure 1.

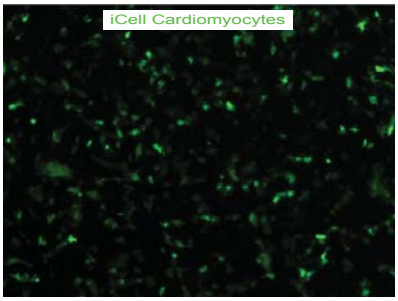
siRNA Transfection. iCell Cardiomyocytes (Cellular Dynamics, #CMC-100-010-001) were transfected with siRNA oligonucleotides to knock down GAPDH (2). iCell Cardiomyocytes were plated in 12-well plates and cultured for 7 days according to the User's Guide. TransIT-TKO Transfection Reagent (Mirus Bio, #MIR 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).

Reporter Construct Transfection. Activation of the cAMP response element (CRE) was measured by transiently transfecting iCell Cardiomyocytes (Cellular Dynamics, #CMC-100-010-001) 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 LTX and 0.2 µl/well of Plus Reagent (Life Technologies, #15338). At 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.

Results & Discussion

Methods commonly used to transfect cell lines often fail when applied to primary cells. iPSC-derived cells recapitulate human biology and are amenable to transfection methods. Transfection of iCell Cardiomyocytes, iCell Neurons, and iCell Endothelial Cells was evaluated with plasmid DNA using multiple commercially available lipid-based transfection reagents (Figure 1). The transfection conditions for each cell type were optimized with different amounts of DNA and transfection reagent according to individual manufacturer's instructions. iCell Cardiomyocytes were tested at days 4 and 12 post-plating, providing transfection conditions for short- and long-term cultures (Figures 1 and 3). iCell Neurons and iCell Endothelial Cells were transfected at day 1 post-plating, enabling a 3-day protocol. Using optimized conditions (Figure 1D), high transfection efficiencies and low cytotoxicities were achieved using the iCell products.

A



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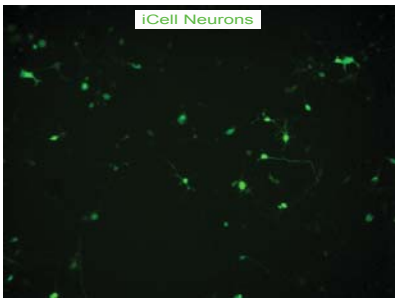

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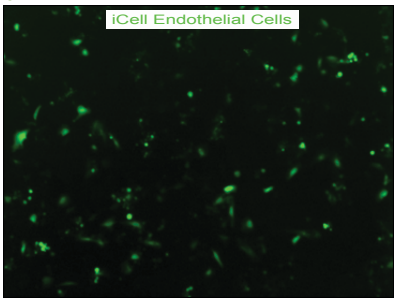
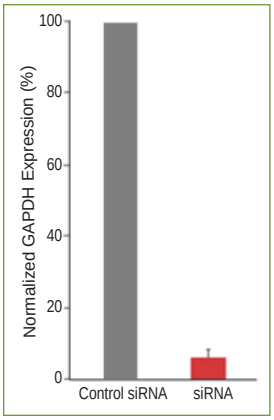


D

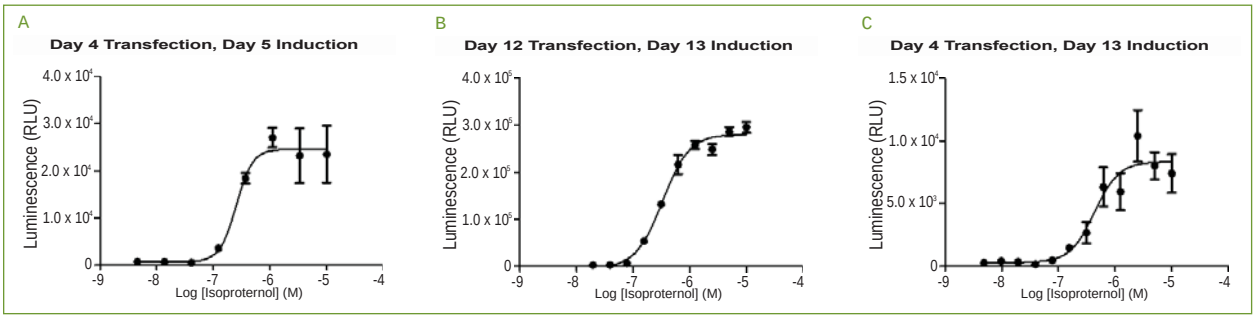
Cells	Culture Conditions (cells/well of 96-well plate)	Reagent(s)	Day of Transfection (days post-plating)	Transfection Duration (hr)	Reagent (µl/well)	DNA (µg/well)	Ratio (reagent:DNA)	Day of Analysis (days post-plating)	Efficiency (% GFP+ Cells)
iCell Cardiomyocytes	20,000	Gene Jammer	4	18	0.3	0.2	1.5:1	6	~40%
		Lipofectamine LTX + Plus	4	18	0.4 LTX + 0.2 Plus	0.2	2:1:1	6	~40%
			12	18	0.4 LTX + 0.2 Plus	0.2	2:1:1	14	~40%
iCell Neurons	40,000	Xfect	1	4	0.1	0.6	1:6	3	~40%*
		Lipofectamine 2000	1	24	0.4	0.4	1:1	3	~25%
iCell Endothelial Cells	8,000 (passage 2)	Lipofectamine LTX + Plus	1	4	0.6 LTX + 0.1 Plus	0.1	6:1:1	3	~25%
		Transit2020	1	4	0.6	0.1	6:1	3	~30%

▲ **Figure 1: Transfection of iCell Products Using Plasmid DNA**
(A - C) Fluorescent microscopy shows GFP-expression of iCell Cardiomyocytes, iCell Neurons, and iCell Endothelial Cells on the day of analysis (100X total magnification). (D) The table lists comparative data of transfection conditions using different transfection reagents. An asterisk (*) denotes detection of cytotoxicity.

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: Transfection of iCell Cardiomyocytes Using siRNA**
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: Long-term Luciferase Reporter Activity in Transiently Transfected iCell Cardiomyocytes**
iCell Cardiomyocytes were transfected transiently with a CRE-responsive luciferase reporter construct at (A, C) 4 days and (B) 12 days post-thaw. The β-adrenergic pathway was stimulated with isoproterenol at (A, B) day 1 and (C) day 9 post-transfection, and luciferase activity was analyzed 24 hours post-treatment (mean ± SEM, n = 3). EC_{50} values for isoproterenol determined in the representative experiments were (A) 0.2 µM, (B) 0.3 µM, and (C) 0.4 µM.

Using reporter gene constructs and a relevant cellular model is particularly important when interrogating endogenous signaling pathways. iCell 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 a CRE-responsive luciferase reporter construct that allows for monitoring the β-adrenergic receptor signal transduction pathway. 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 (Figure 3). These data illustrate that the CRE-luciferase construct was transfected efficiently, the endogenous signaling pathway was induced, and the signaling activity was maintained over time in culture.

Conclusion

iCell products can be transfected efficiently with commercially available transfection reagents with limited cytotoxicity. This distinct advantage of human iPSC-derived cells over primary cells, combined with the functional properties of iCell products, offers easy-to-use cellular systems to perform exogenous gene delivery, endogenous gene modulation, and induction of specific cellular responses. The ease of culture of iCell 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, using iCell Cardiomyocytes, iCell Neurons, and iCell Endothelial Cells.

References

1. Cellular Dynamics International, Inc. (2012) Published Research. www.cellulardynamics.com/pubslist/.
2. Cellular Dynamics International, Inc. (2012) iCell Cardiomyocytes - Using Transfection for siRNA Delivery Application Protocol. www.cellulardynamics.com/lit/.

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