Cellular Dynamics international

Application Note

## iCell® Neural 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 terminally differentiated human iPS cell-derived GABAergic cortical neurons, iCell® GABANeurons, midbrain dopaminergic neurons, iCell DopaNeurons, glutamatergic neurons, iCell GlutaNeurons, and spinal motor neurons, iCell Motor Neurons. These cells provide unprecedented access to human neural cell types as in vitro model systems, having demonstrated biological relevance and functional utility in various drug screening and toxicity applications.[1]

This Application Note highlights how iCell GABANeurons and iCell DopaNeurons are compatible with commercially available transfection reagents, thus enabling the regulation of specific target genes for numerous applications in drug discovery research and for target-based and phenotypic-based screens. Transfection reagents were evaluated for delivery of plasmid DNA and small interfering RNA (siRNA) oligonucleotides.

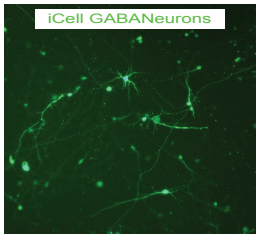
## Methods

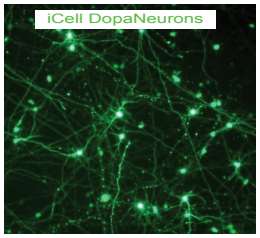
**Plasmid DNA Transfection.** iCell GABANeurons and iCell DopaNeurons (Cellular Dynamics, Cat. No. R1013 and R1032, respectively) were transfected using ViaFect Transfection Reagent (Promega, Cat. No. E4981). The cells were plated in 96-well cell culture plates and cultured for 4 or 21 days according to the relevant Application Protocol.[2,3] Plasmid DNA encoding green fluorescent protein (pZsGreen1-N1, Clontech, Cat. No. 632448) was delivered into the cells according to the ViaFect manufacturer's instructions. The transfection efficiency was assessed at 72 hours post-transfection by fluorescence microscopy or flow cytometry (represented as a percentage of GFP-expressing cells). Optimal transfection and analysis parameters are provided in Figure 1.

**siRNA Transfection.** iCell GABANeurons were transfected with siRNA oligonucleotides to knock down GAPDH.[4] iCell GABANeurons were plated in 96-well cell culture plates and cultured for 4 days according to the User's Guide. The cells were incubated with Accell siRNA (Thermo Fisher Scientific), and the effect of the RNAi-mediated knockdown of GAPDH was measured at 72 hours post-transfection. The expression levels of GAPDH (relative to 18s RNA) in test samples were normalized to controls (cells transfected with a non-targeting siRNA).

## Results & Discussion

Methods commonly used to transfect cell lines often fail when applied to primary neural cells. iPSC-derived cells recapitulate human biology and are amenable to transfection methods. Transfection of iCell GABANeurons and iCell DopaNeurons was evaluated with plasmid DNA using a commercially available lipid-based transfection reagent (Figure 1). The transfection conditions for each cell type were optimized with different amounts of DNA and transfection reagent according to the manufacturer's instructions. iCell GABANeurons and iCell DopaNeurons were transfected at day 4 post-plating and analyzed at 72 hours post-transfection enabling a short assay protocol. Using optimized conditions (Figure 1C), high transfection efficiencies and low cytotoxicities were achieved in both cell types. The same transfection conditions were tested at day 21 post-plating where iCell GABANeurons achieved a similar level of efficiency.

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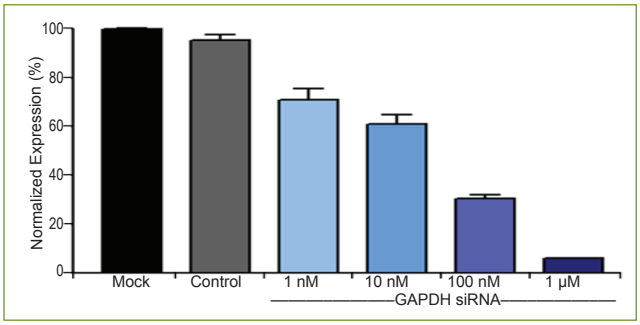
| Cells | Day of Transfection (days post-plating) | ViaFect Transfection Reagent (µl/well) | DNA (µg/well) | Ratio (reagent:DNA) | Efficiency (% GFP+ cells) |
|---|---|---|---|---|---|
| iCell GABANeurons | 4 | 0.6 | 0.1 | 6:1 | 30% |
| | 21 | 0.2 | 0.1 | 2:1 | 30% |
| iCell DopaNeurons | 4 | 0.4 | 0.1 | 4:1 | 50% |
| | 21 | 0.4 | 0.1 | 4:1 | 25% |

▲ **Figure 1: Transfection of iCell GABANeurons and iCell DopaNeurons Using Plasmid DNA**
*(A - B) Fluorescent microscopy shows GFP-expression on the day of analysis (20X and 40X magnification, respectively). (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 GABANeurons 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 GABANeurons as demonstrated by the effective knockdown of target gene expression.




▲ **Figure 2: Transfection of iCell Neurons Using siRNA**
*iCell Neurons exhibit a reduction of GAPDH mRNA levels following transfection using target-specific siRNA compared to the control siRNA (mean ± SEM, n = 3).*

## Conclusion

iCell GABANeurons and iCell DopaNeurons can be transfected efficiently and with limited cytotoxicity using commercially available transfection reagents. This advantage over primary cells, along with the functional properties of human iPSC-derived iCell neural products, provides relevant cellular systems for exogenous gene delivery, endogenous gene modulation, and induction of specific cellular responses.[5] In addition, the ease-of-culture of iCell neural products and the stability over time of their cellular functionality in culture allow for short- and long-term studies. These capabilities enable a wide range of academic and pharmaceutical research applications, in particular high-throughput screening and drug discovery.

## References

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## For More Information

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