Cellular Dynamics international

iCell® Neurons
Application Protocol

Silencing Gene Expression:
siRNA Delivery by Transfection

Introduction

Delivery of exogenous small interfering RNA (siRNA) in cultured cells is an effective method to modulate target gene expression via RNA interference (RNAi). However, genetic manipulations in primary neuronal cultures are especially inefficient and often toxic. In fact, neurons are considered one of the most difficult and resistant cell types for introduction of siRNA oligonucleotides.

Several siRNA delivery systems and reagents are available depending on the cell type, cell culture preparation, and desired level of target gene silencing. The protocol presented here demonstrates siRNA delivery by transfection for efficient GAPDH gene silencing in iCell® Neurons using the Accell siRNA technology in 96-well cell culture plates. This Application Protocol serves as a guide for delivery of other Accell siRNAs for use in different plate formats and endpoint readouts.

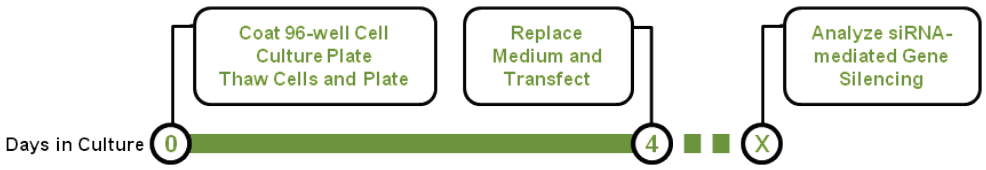
Required Equipment and Consumables

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

Item	Vendor	Catalog Number
Equipment		
Multichannel Pipettors	Multiple Vendors	
Quantitative Real-time PCR (qRT-PCR) Instrument	Multiple Vendors	
Consumables		
iCell Neurons Kit	Cellular Dynamics International (CDI)	NRC-100-010-001
1.5 ml RNAse-free Tubes	Multiple Vendors	
5X siRNA Buffer	Thermo Scientific	B-002000-UB-100
96-well Cell Culture Plates	Multiple Vendors	
Accell siRNA Delivery Media	Thermo Scientific	B-005000-500
Accell siRNA Oligonucleotides (siRNA)	Thermo Scientific	
Nuclease-free Water	Multiple Vendors	
RNAse-free Water	Multiple Vendors	

Workflow

iCell Neurons are thawed and plated into a 96-well cell culture plate previously coated with poly-L-ornithine and laminin solutions. On day 4 post-plating, medium is replaced, and cells are transfected. The siRNA-mediated gene silencing is analyzed subsequently at the optimal post-transfection timepoint for the target gene and endpoint assay.



Methods

Culturing iCell Neurons

1. Coat a 96-well cell culture plate with a base layer of 0.01% poly-L-ornithine solution and a top coating of a 3.3 µg/ml laminin solution according to the iCell Neurons User's Guide.
2. Prepare Complete iCell Neurons Maintenance Medium (Complete Maintenance Medium) according to the User's Guide.
3. Thaw iCell Neurons according to the User's Guide.
4. Remove a sample of the cell suspension and count the viable neurons using a hemocytometer.
5. Further dilute the cell suspension in Complete Maintenance Medium to 400,000 cells/ml.
6. Aspirate the laminin solution from the 96-well cell culture plate. Immediately add 100 µl/well of the cell suspension (40,000 cells/well).
7. Incubate in a cell culture incubator at 37°C, 5% CO_2. Maintain the neurons according to the User's Guide until ready to perform the transfection.

Transfecting iCell Neurons with siRNA

The following procedure details transfection of iCell Neurons cultured on day 4 post-plating. The volumes indicated are per well of a 96-well cell culture plate. Scale volumes appropriately for multiple wells or for other well formats.

1. Warm the Accell siRNA Delivery Media to room temperature.
2. Dilute 5X siRNA Buffer in RNAse-free water to achieve a 1X siRNA Buffer.
3. Briefly centrifuge each tube containing siRNA to ensure the pellet is at the bottom of the tube.
4. Reconstitute siRNA in 1X siRNA Buffer to achieve a 100 µM stock solution.

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5. Dispense 125 µl of Accell siRNA Delivery Media in a 1.5 ml RNAse-free tube.
6. Add 1.25 µl of 100 µM siRNA stock solution to achieve a 1 µM siRNA delivery mix. Mix gently.

   Note: *Store the remaining 100 µM siRNA stock solution at -20°C.*
7. Remove the 96-well cell culture plate containing iCell Neurons from the incubator.
8. Aspirate Complete Maintenance Medium from the 96-well cell culture plate and replace with 100 µl/well of 1 µM siRNA delivery mix.
9. Incubate in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.
10. Aspirate 1 µM siRNA delivery mix and replace with 100 µl/well of fresh Complete Maintenance Medium.
11. Incubate in a cell culture incubator at 37°C, 5% CO_2. Maintain iCell Neurons according to the User's Guide until the optimal timepoint to analyze the siRNA-mediated gene silencing is attained.

Measuring siRNA-mediated Gene Silencing in iCell Neurons

The optimal post-transfection timepoint at which to measure the RNAi-mediated effect depends on the target gene, siRNA, and endpoint assay. In a representative experiment, GAPDH mRNA silencing in iCell Neurons (Figure 1) was measured on day 7 post-plating (day 3 post-transfection) using the TaqMan Gene Expression Cells-to-C_T Kit (Life Technologies, Cat. No. 4399002M) according to the manufacturer's instructions.

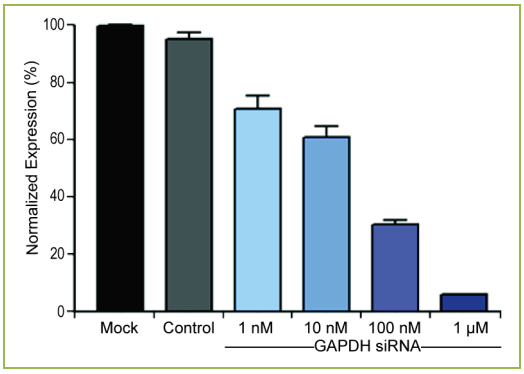

Figure 1: RNAi-mediated Gene Silencing in iCell Neurons
On day 4 post-plating, iCell Neurons were transfected with Accell siRNA Delivery Media only (mock), a control (scrambled) siRNA in Accell siRNA Delivery Media, or a titration of GAPDH siRNA in Accell siRNA Delivery Media. On day 7 post-plating (day 3 post-transfection), GAPDH mRNA levels were measured relative to 18s rRNA levels and compared to the mRNA levels obtained following transfection of the control siRNA (mean ± SEM, n = 3 independent siRNA delivery mixes).

Summary

iCell Neurons provide an in vitro test system that recapitulates native human neurobiology while the Accell siRNA technology offers an efficient method for siRNA transfection experiments. The methods presented here highlight the specificity with which specific gene function can be examined precisely in human neurons via RNAi. Furthermore, the data suggest that the impact of RNAi-mediated knockdown of specific target genes will be effective when monitored through other endpoint assays.

Notes

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