Using Transfection for siRNA Delivery

Introduction

Delivery of exogenous small interfering RNA (siRNA) in cultured cells is an effective method to modulate target gene expression via RNA interference (RNAi). 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 has demonstrated utility in delivering siRNA for efficient GAPDH gene silencing in iCell® Cardiomyocytes using TransIT-TKO Transfection Reagent in 12-well cell culture plates at 7 days post-plating and should serve as guide to deliver other siRNAs using other cell culture vessel formats at different timepoints post-plating.

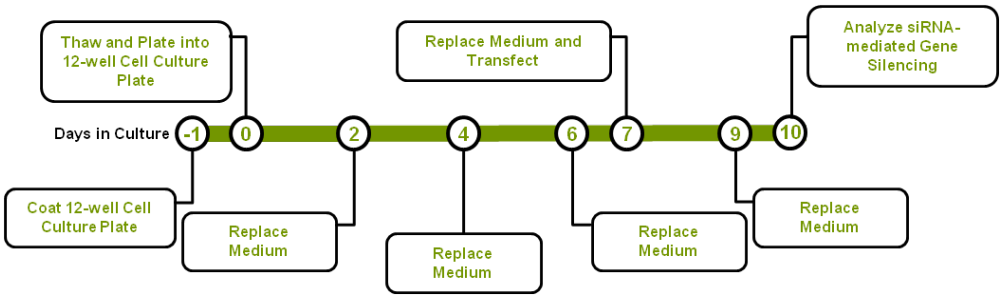
Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Cardiomyocytes User's Guide and iCell Cardiomyocytes Application Protocol: Extracting Total RNA.

Item	Vendor	Catalog Number
Equipment		
Quantitative Real-time PCR (qRT-PCR) Thermal Cycler	Multiple Vendors	
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-110-001 CMC-100-110-005 CMC-100-010-001 CMC-100-010-005
12-well Cell Culture Plates	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14190
Fibronectin	Roche Applied Science	11051407001
High Capacity cDNA Reverse Transcription Kit	Life Technologies	4368814
Opti-MEM Reduced Serum Medium	Life Technologies	31985-062
qRT-PCR Reagents	Multiple Vendors	
RNAse-free Water	Multiple Vendors	
siRNA Oligonucleotides	Multiple Vendors	
Sterile Water	Multiple Vendors	
TransIT-TKO Transfection Reagent	Mirus Bio	MIR2150

Workflow

iCell Cardiomyocytes are thawed and plated into a 12-well cell culture plate previously coated with fibronectin. On days 2, 4, 6, and 7 post-plating, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium). On day 7 post-plating, cells are transfected with siRNA. On day 9 post-plating, spent medium is replaced with fresh Maintenance Medium. On day 10 post-plating, cells are analyzed for siRNA-mediated gene silencing.



Methods

Culturing iCell Cardiomyocytes

1. Dilute 1 mg/ml of fibronectin solution in sterile D-PBS to a final concentration of 5 µg/ml immediately before use.

   Note: *Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20˚C.*

2. Add 1 ml/well of 5 µg/ml fibronectin solution to a 12-well cell culture plate.
3. Incubate in a cell culture incubator at 37°C overnight.
4. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.
5. Dilute the iCell Cardiomyocytes cell suspension in iCell Cardiomyocytes Plating Medium to 150,000 plated cells/ml. See the iCell Cardiomyocytes User's Guide for instructions to calculate the *Target Plating Density* based on *Plating Efficiency*.
6. Aspirate the fibronectin solution. Immediately add 1 ml/well of the cell suspension (150,000 plated cells/well).
7. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO_2.
8. Maintain the cardiomyocytes according to the User's Guide until ready to perform the transfection.

Transfecting iCell Cardiomyocytes with siRNA

The following procedure details transfection of iCell Cardiomyocytes cultured for 7 days in 12-well cell culture plates. The volumes indicated are per well of a 12-well cell culture plate. Scale volumes appropriately for other vessel formats.

Notes

Notes

1. Replace the medium with 1 ml of fresh iCell Cardiomyocytes Maintenance Medium (Maintenance Medium).
2. Equilibrate the TransIT-TKO Transfection Reagent at room temperature. Vortex gently before use.
3. Reconstitute siRNAs (control siRNA and targeting siRNA) in RNAse-free water at 10 µM. Store the 10 µM siRNA stock solution at -20˚C.
4. Place 100 µl of Opti-MEM Reduced Serum Medium in a 1.5 ml RNase-free tube.
5. Add 4 µl of TransIT-TKO Transfection Reagent. Mix gently.
6. Add 5.5 µl of 10 µM siRNA stock solution to obtain a final concentration of 50 nM/well. Mix gently.
7. Incubate at room temperature for 15 - 30 minutes to allow TransIT-TKO:siRNA complexes to form.
8. Dispense the TransIT-TKO:siRNA complexes drop-wise across the surface of the well.
9. Rock the 12-well cell culture plate gently back-and-forth and side-to-side to distribute evenly the TransIT-TKO:siRNA complexes.
10. Incubate in a cell culture incubator at 37°C, 7% CO_2 for 48 hours.
11. Replace the medium with 1 ml of fresh Maintenance Medium.
12. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO_2.
13. Maintain the cardiomyocytes according to the User's Guide until ready to measure the siRNA-mediated gene silencing.

Measuring siRNA-mediated Gene Silencing in iCell Cardiomyocytes

The optimal post-transfection timepoint at which to measure the RNAi effect of siRNAs depends on the target gene, siRNAs, and phenotypic assay. GAPDH mRNA silencing in iCell Cardiomyocytes (Figure 1) was measured 72 hours post-transfection using the following procedure.

1. Extract total RNA from the transfected cardiomyocytes according to the iCell Cardiomyocytes Application Protocol: Extracting Total RNA.
2. Reverse transcribe mRNA into cDNA according to the manufacturer's instructions for the cDNA Reverse Transcription Kit.
3. Store the cDNA at -20°C.
4. Perform qRT-PCR to measure the expression of the target gene.

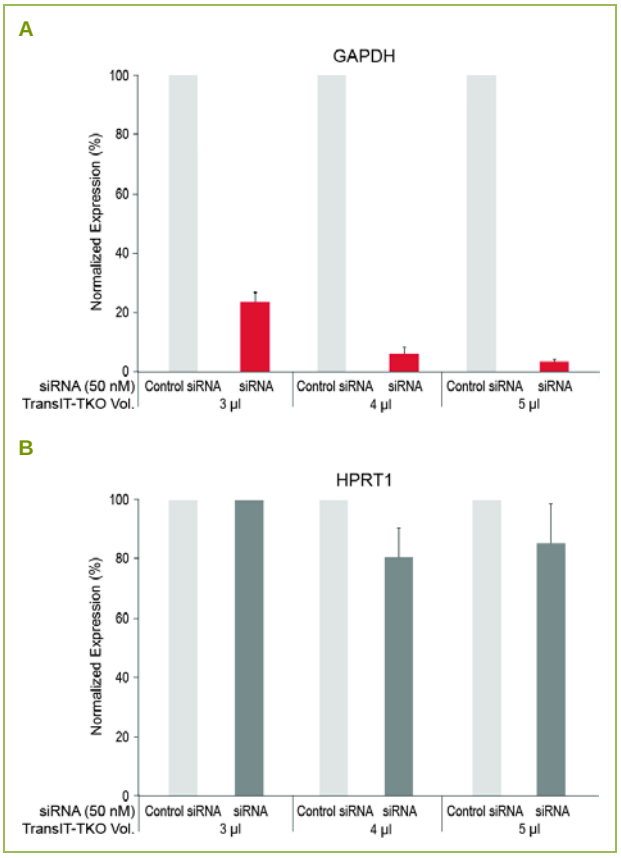


Figure 1: siRNA-mediated Gene Silencing in iCell Cardiomyocytes
Panels A and B show the effect of GAPDH-targeted siRNA on GAPDH (targeted) and HPRT1 (non-targeted) mRNA expression, respectively. iCell Cardiomyocytes were cultured for 7 days in a 12-well cell culture plate before transfection with either control (scrambled) or GAPDH siRNA (sense: GCUCAUUUCCUGGUAUGACUU; antisense: GUCAUACCAGGAAAUGAGCUU) using TransIT-TKO Transfection Reagent (3 - 5 µl/well). 72 hours post-transfection, the GAPDH and HPRT1 mRNA levels were measured relative to 18s rRNA levels and normalized to the mRNA levels obtained following transfection of the control siRNA in each experiment (mean ± SEM, n = 3 independent transfection complexes).

Summary

iCell Cardiomyocytes provide an in vitro test system that recapitulates native human cardiac myocyte physiology and function while TransIT-TKO Transfection Reagent offers an efficient method for siRNA transfection experiments. The methods and data presented here highlight the ease of use and specificity with which specific gene and protein function can be precisely examined in human cardiomyocytes via RNAi.

Version: October 2013
AP- CMCRNAI131028

Notes