APPLICATION NOTE

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Dharmacon™ Accell™ siRNA reagents: achieving long-term gene silencing

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Abstract

Developing a thorough understanding of a gene's contribution to a particular phenotype is problematic when the protein has an extended half life or the phenotype under study requires days to materialize. Under these circumstances, reagents that induce long-term gene silencing are needed to sufficiently deplete internal cellular stores for extended periods of time. To address this need, we have developed Dharmacon Accell siRNA reagents, with novel modifications enabling uptake by cells without the aid of lipid transfection reagents. This innovative delivery technology allows repeated application of Accell siRNA reagents to provide extended duration gene knockdown with only minimal effects on cell viability and the innate immune response. These attributes greatly broaden the range of biological questions and cell types that can be investigated by researchers using RNA interference (RNAi).

Introduction

Over the years, gene silencing by RNAi has relied heavily on the use of cationic lipid-based delivery reagents for transfecting cells with small interfering RNAs (siRNAs). In many cases, lipid-based transfection is sufficient and provides efficient levels of gene knockdown. Yet in a significant fraction of cases, cells are either refractory to lipid-mediated delivery of siRNAs or aversely sensitive to the presence of lipids.

The Accell siRNA product line represents a recently developed technology that promotes delivery of siRNA independent of viral vectors or lipid-based transfection reagents (also referred to as "passive delivery") to virtually any cell type. Through the incorporation of a unique combination of chemical and bioinformatic enhancements that increase functionality, stability, and lipid-independent delivery, Accell siRNAs can be used in a wide range of human, mouse, and rat cell lines that have been traditionally identified as difficult to transfect. (Appendix I, see back page for a list of cells into which Accell siRNAs have been successfully tested.) This novel delivery technology permits gene silencing with minimal off-target events (as assessed by genome-wide profiling) has limited repercussions on cell viability, and triggers a negligible innate immune response as assessed by quantitation of nine inflammatory response proteins using the SearchLight™ Array platform (Human Inflammatory Cytokine Array 1; Thermo Scientific Cat. # 84619). Thus, unlike electroporation, which is historically the first alternative delivery method for cells found to be averse to lipid-based transfection reagents, Accell siRNA reagents can be employed without significant effects on cell physiology.

Over the course of investigations, it is often necessary to knock down gene expression for extended periods of time to accurately assess the contribution of a particular protein to a given biological event. Long-term reduction of gene expression can be problematic, particularly in cases where there are large intracellular reservoirs of the target or the half-life of the protein is drawn out. In some instances, researchers have attempted multiple, consecutive lipid-mediated transfections of siRNAs into cultured cells. Unfortunately, this approach is frequently cytotoxic and can induce brief (24-72 hours) changes in the state of the innate immune response. To overcome these barriers, DNA-mediated RNAi, commonly exploiting viral delivery platforms, has been adopted. This approach can be successful, but is costly and requires significant development time. For a solution that requires neither lipid transfection nor viral delivery, this Application Note provides details of how Accell siRNA reagents may be applied to provide long-term silencing of gene expression.

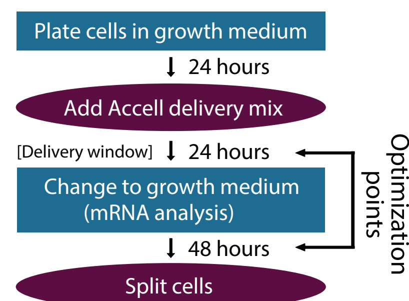

Figure 1. Repeated application workflow for inducing long-term gene silencing in cultured cell lines. Following the initial plating in growth medium (medium plus serum, supplements and antibiotics), cells are treated with Accell siRNA resuspended in the Accell Delivery Media. After 24-48 hours, the delivery mix is replaced with complete medium until cells are confluent, upon which the cultures are split and Accell delivery mix is re-applied.

Workflow for long-term gene knockdown using Accell siRNA

Figure 1 provides an overview of how Accell siRNA can be used to induce gene knockdown for extended periods of time. The day after cells are trypsinized, counted and plated, the growth medium used to culture cells (containing serum, supplements and antibiotics) is replaced with Accell Delivery Media (Cat. #B-005000-100, B-005000-500) containing Accell siRNA targeting the gene of interest (referred to as the Accell delivery mix). Passive transfection then proceeds for 24-48 hours, whereupon the Accell delivery mix is exchanged for growth medium, and cells are cultured for an additional period (generally 24-48 hours). Prior to reaching confluency, cultures are split, re-plated, and treated again using the same protocol described in the previous round. If cells require additional time prior to splitting, they may be treated again with Accell delivery mix without splitting. This may vary depending upon the cell type; however, the cycling between growth medium and Accell delivery mix can be performed with little affect on cell viability to provide long-term gene knockdown in your cell type of choice.

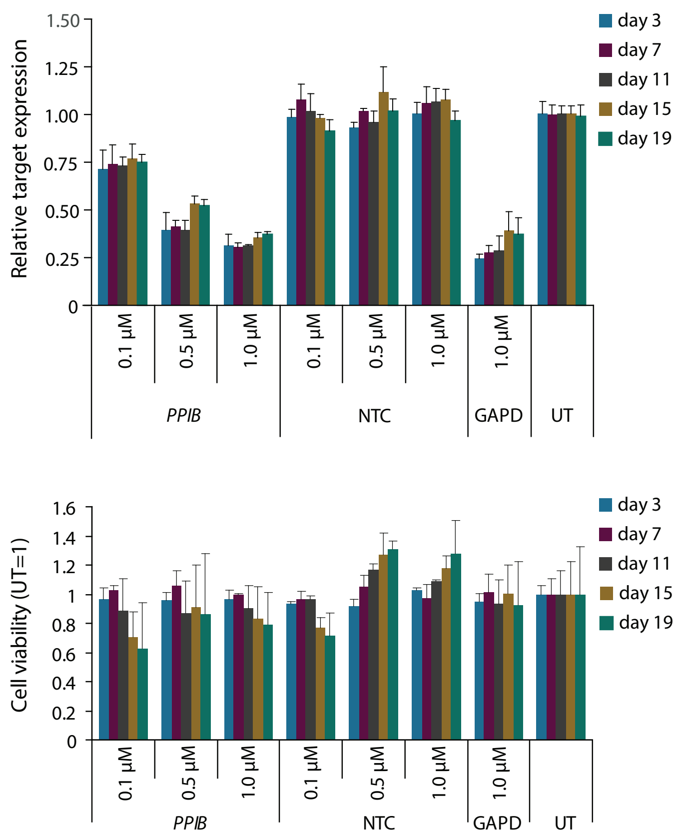

Figure 2. Demonstration of long-term gene knockdown using Accell siRNA technology. SHSY5Y neuroblastoma cells were plated at a density of 5,000 cells per well (96-well plate) and cultured overnight. The following day, the complete medium was exchanged with the Accell delivery mix containing Accell siRNA targeting housekeeping gene PPIB at 0.1, 0.5 and 1 µM or GAPDH at 1 µM. Accell Non-targeting siRNA #1 (NTC) was also used at 0.1, 0.5 and 1 µM concentrations at every passage. After 48 hours, the overlying delivery mix was replaced with complete medium. Target knockdown (A.) and cell viability (B.) was assessed on days 3, 7, 11, 15, and 19 using bDNA and Thermo Scientific™ alamarBlue™ (BioSource, Intl), respectively. (UT – Untreated control cells that have gone through the same procedure of medium changes as Accell-treated cells).

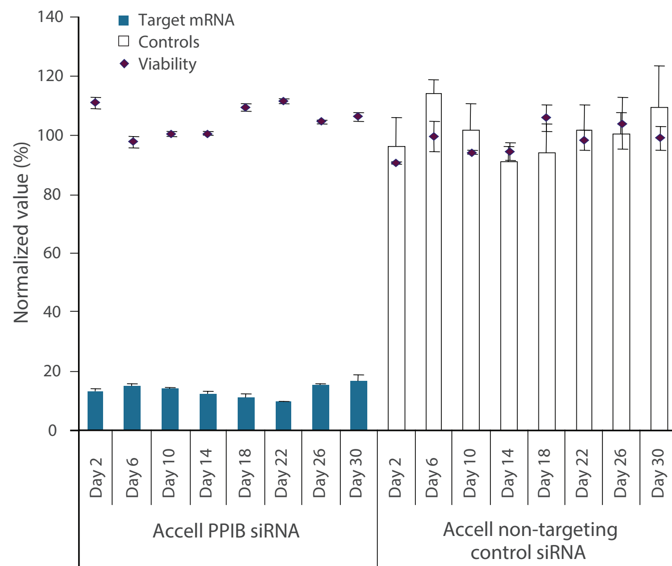

Figure 3. Demonstration of long-term gene knockdown using Accell siRNA technology. HeLa cells were plated at a density of 10,000 cells per well (96-well plate) and cultured overnight. The following day, the complete medium was exchanged with the Accell delivery mix containing 1 µM Accell siRNA targeting the housekeeping gene PPIB. Dharmacon Accell Non-targeting siRNA #1 was also used at 1 µM concentrations at every passage. Following 24 hours of treatment, the delivery mix was replaced with complete medium. This workflow (Figure 1) was repeated, and target knockdown and cell viability were assessed on days 2, 6, 10, 14, 18, 22, 26, and 30 using bDNA and alamarBlue™, respectively.

Demonstration of long-term gene knockdown using Accell sirna targeting *PPIB*

To demonstrate the feasibility of providing long-term gene knockdown using Accell technology, the workflow described above was employed to target the housekeeping gene PPIB (Cyclophilin B, NM_000942) in SH-SY5Y neuroblastoma cells. Accell siRNA targeting PPIB was delivered at one of three concentrations (100 nM, 500 nM, or 1 µM) and the levels of mRNA knockdown were assessed using branched DNA (bDNA; Panomics) on days 3, 7, 11, 15, and 19. Accell Non-targeting siRNA #1 (Cat. #D-001910-01) was included to assess the specificity and overall effects of the treatment on cell viability. Finally, at each passage, untreated cells subjected to medium changes and splitting were treated with Accell siRNA targeting GAPDH as a control for transfectability of the cells.

The results of these experiments demonstrate the efficacy of Accell technology in providing long-term gene knockdown (Figure 2). Accell siRNA-mediated gene knockdown of PPIB exhibited a dose-dependent response, with 1 µM concentrations providing greater than 70% reduction in gene expression in SH-SY5Y cells. Equally important, knockdown was specific while cell viability generally remained at 80% over the course of the 5 passages (19 days). The repeated application workflow was also optimized for HeLa cells, resulting in a 30-day duration of silencing (Figure 3).

Compatibility of Accell technology with lipid-mediated delivery

To test whether long-term gene silencing using Accell siRNA was compatible with subsequent lipid-mediated siRNA transfections, HeLa cells treated over the course of 10 days with an Accell PPIB targeting siRNA were thereafter transfected with Dharmacon™ siGENOME™ (unmodified) siRNA targeting GAPDH (Cat. #D-001140-01-05) and delivered using Dharmacon™ DharmaFECT™ 1 Transfection Reagent (Cat. #T-2001-01). The results of these experiments presented within Figure 4, show that passive delivery using Accell siRNA technology is compatible with standard lipid transfection techniques. In cases where cells were treated with both the Accell PPIB siRNA and the siGENOME GAPDH siRNA delivered by DharmaFECT 1, overall knockdown for each gene was 80% or greater (day 13). These studies attest to the flexibility of experimental design when using Accell siRNA reagents and demonstrate the ability to achieve multi-gene knockdown when using Accell siRNA in conjunction with lipid-mediated delivery techniques.

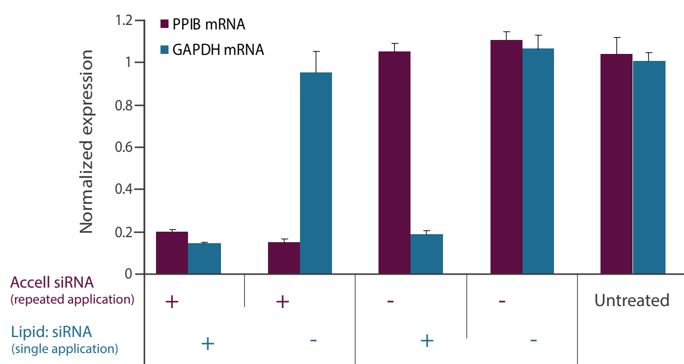

Figure 4. compatibility of Accell siRNA technology with alternative delivery methods. HeLa cells were treated with 1 µM Accell PPIB siRNA over the course of three passages (10 days) as described. On day 10, cells were split, re-plated and transfected with GAPDH-targeting siRNA (100 nM) using Dharmacon DharmaFECT 1 transfection reagent (DF1). Twenty-four hours after lipid-mediated transfection, the expression levels of PPIB and GAPDH were assessed using technologies.

Summary

While application of Accell siRNA reagents for short-term gene silencing requires little optimization, variations should be tested to identify the best protocol for long-term gene silencing in your cell type. Incorporation of controls that allow for assessment of the effects of passive delivery on cell viability and overall physiology are considered prudent to ensure that repeated exposure to Accell delivery mix does not introduce unwanted changes in the cell's phenotypic state.

Long-term gene knockdown is essential when protein stability and/or assay design requires sustained silencing. As demonstrated above, repeated application with the Accell delivery mix resulted in continuous silencing for up to 30 days. The described workflow is relatively non-toxic and compatible with lipid-mediated transfection (thus allowing multi-gene or combinatorial knockdown). This novel passive delivery technology significantly expands the experimental design options of researchers by permitting the use of cells that are historically refractory to lipid-mediated transfection.

Human adherent cell lines (24)					
SH-SY5Y	IMR-32	LAN-5	HEK293T	A549	U-87 MG
LNCaP	A-375	HeLa	HeLa S3	MIA PaCa-2	HEK293
MCF7	MCF 10A	SK-BR3	OVCAR-3	NCI/ADR-RES	U2OS
Huh7	GTM-3	DU 145	HT-1080	HepG2	DLD-1
Mouse and rat cell lines (7)					
NIH/3T3	ES-D3	PC-12	3T3-L1	H9c2	C2C12
Rat2					
Human suspension cell lines (4)					
Jurkat	IM-9	THP-1	PBMC		
Human differentiated stem cells (2)					
Osteoblasts and adipocytes derived from hMSC					
Human, mouse, and rat primary cells (9)					
HUVEC	HUASMC	hMSC	Primary human astrocytes	primary human keratinocytes	primary human T-cells
Primary mouse hepatocytes		Primary rat cortical neurons		primary rat striatum neurons	
Cell types that failed Dharmacon Accell sirna application (0)					

Please check https://dharmacon.horizondiscovery.com/rnai/sirna/accell/#all for the most up-to-date information.

If you have any questions

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