Innovative technology that enables RNAi in difficult to transfect cells

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Abstract

Delivery remains one of the last barriers for applying RNA interference (RNAi) in clinically relevant cell lines. Investigations at Dharmacon have led to the development of an innovative molecule for delivery in a wide variety of cell types. These modified siRNAs have been found to effectively silence target genes in cell types that are typically difficult to transfect using standard delivery methods. We present data for multiple cell types including SH-SY5Y (neuroblastoma), Jurkat (T-cells), and primary neurons. This technology, Dharmacon™ Accell™ siRNA reagents, allows for functional genomic studies in pertinent cell types. Moreover, in some instances, cells can be continuously dosed with these siRNAs, thus enabling knockdown of any target gene of interest for extended durations.

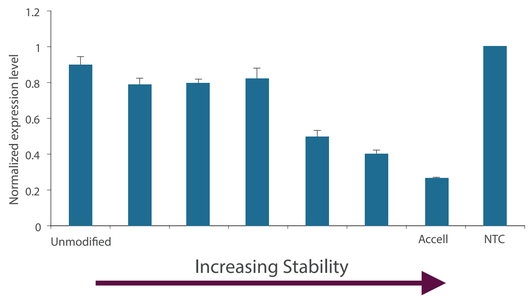
Development of a self-delivering siRNA

Considerations:
- Stabilized siRNA molecule due to lack of encapsulation
- Minimize non-specific delivery response
- Potent knockdown: algorithm validation in multiple cell lines

Protocol

Applications:
- Difficult-to-transfect cell lines
- Extended duration knockdown
- Phenotype using High Content Analysis

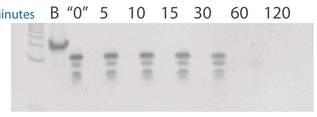
Increased stability for improved efficacy



Accell siRNAs (1 µM with various chemical modification patterns targeting cyclyphilin B were delivered into Hela cells (plated at 2500 cells/well) in Accell Delivery Media. Cyclophilin B expression was determined by QuantiGene branched DNA assay (Panomics) 72 hours after delivery.

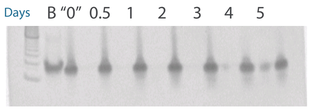
Unmodified

Minutes B "0" 5 10 15 30 60 120



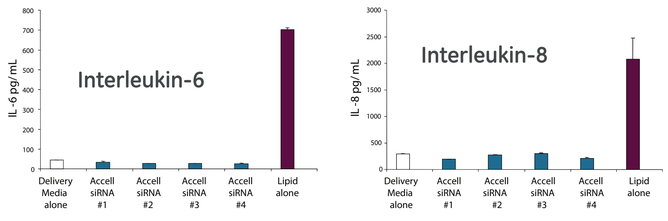
Accell siRNA

Days B "0" 0.5 1 2 3 4 5



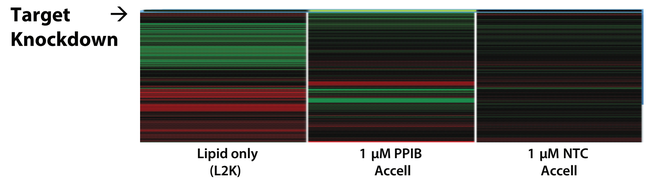
Unmodified and Accell siRNA was mixed with 100% human serum for the indicated times. The reaction was stopped with 40 mM EDTA and stored at -20 °C. The samples were run on a 20% TBE acrylamide gel and stained with ethidium bromide. Lane 1 is a 10 bp DNA ladder (Invitrogen); Lane B is siRNA in buffer alone.

Accell siRNA does not elicit an inflammatory response



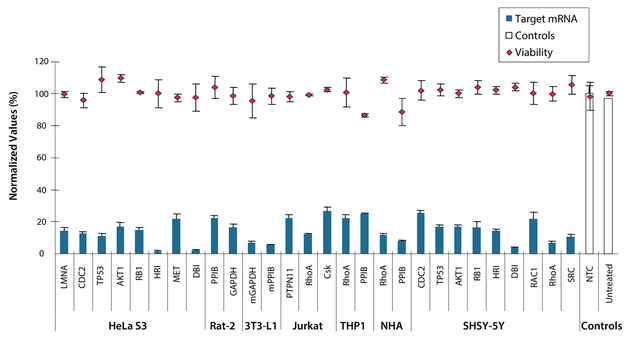
HeLa S3 cells were treated with either Transfection Reagent (lipid) alone (DharmaFECT 1 Transfection Reagent), 1 µM Accell siRNA (either #1, 2, 3 or 4) targeting diazepam binding inhibitor (DBI) or Accell Delivery Media alone. 72 hours after transfection, the cellular supernatant was analyzed for the cytokine IL-8 or IL-6 using the SearchLight array platform.

Accell siRNA reduces non-specific delivery effects



Total RNA from HeLa cells treated with Lipofectamine™ 2000 (L2K; Invitrogen) alone, 1 µM Accell Cyclophilin B Control siRNA or Accell Non-targeting Control (NTC) siRNA #1 was measured by microarray analysis (Agilent Whole Human Genome Array, 22K format).

Potent siRNA – algorithm validation in multiple cell lines

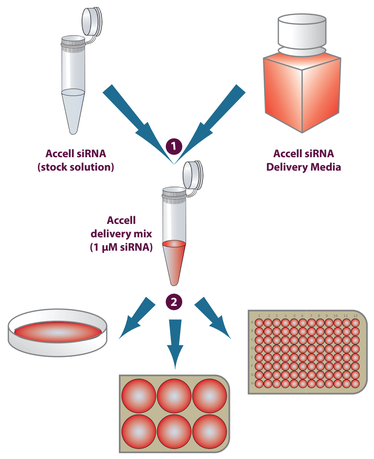


HeLa S3, Rat-2, 3T3-L1, Jurkat, THP-1, NHA, and SH-SY5Y cells were treated with 1 µM Accell SMARTpool siRNA targeting various genes or Accell Non-targeting Control (NTC). At 72 hours, mRNA expression was determined by QuantiGene branched DNA assay (Panomics) and cell viability was determined by alamarBlue (Thermo Fisher Scientific).

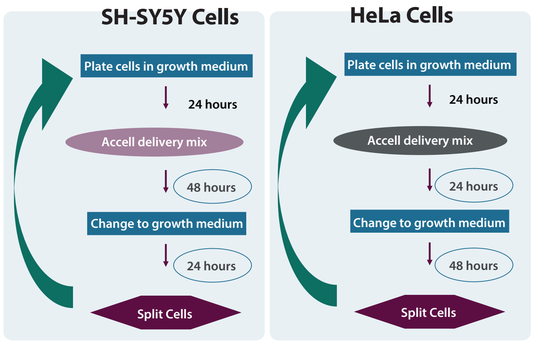
A straightforward protocol for reproducibility and ease-of-use

1. Combine Accell siRNA with Accell Delivery Media or other low- or no-serum medium
2. Add to cells and incubate a minimum of 72 hours

Serum components bind Accell siRNA and inhibit its passive delivery properties. If cells demonstrate sensitivity to the serum-free Accell application conditions, growth medium may be added back after 48 hours of treatment.

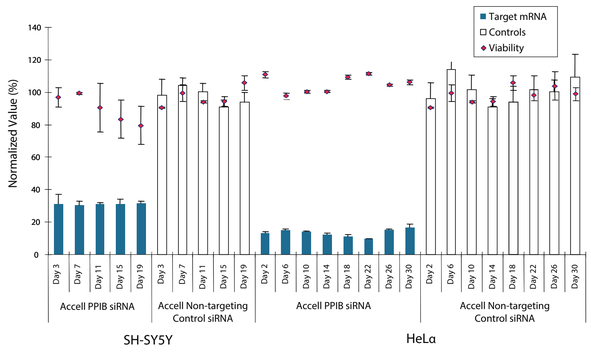


Extended knockdown - Repeated application of Accell siRNA



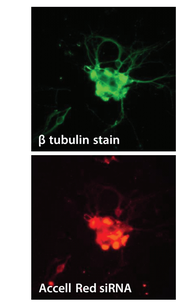
Conditions were optimized for HeLa and SH-SY5Y cells for repeated dosing with Accell delivery mix (Accell siRNA in Accell Delivery Media) alternated with growth media to extend the duration of target gene knockdown.

Target knockdown for up to 30 days



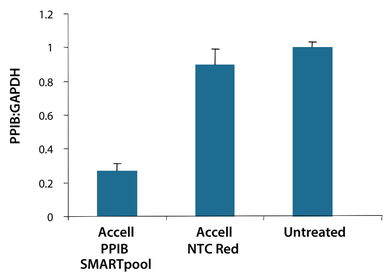
HeLa cells showed sustained knockdown for 30 days (9 passages), SH-SY5Y for 20 days (5 passages). At 24 hours (HeLa) or 48 hours (SH-SY5Y) post-transfection Cyclophilin B expression was determined by QuantiGene branched DNA assay (Panomics) and cell viability was determined by alamarBlue (Thermo Fisher Scientific).

Accell siRNA delivery into primary neurons

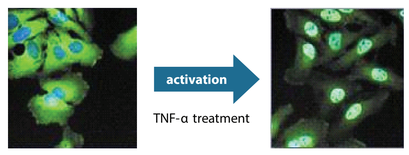


1 µM Accell Red Non-targeting Control siRNA - imaged at 72 hours

Knockdown of target gene by ~ 75% by Accell siRNA



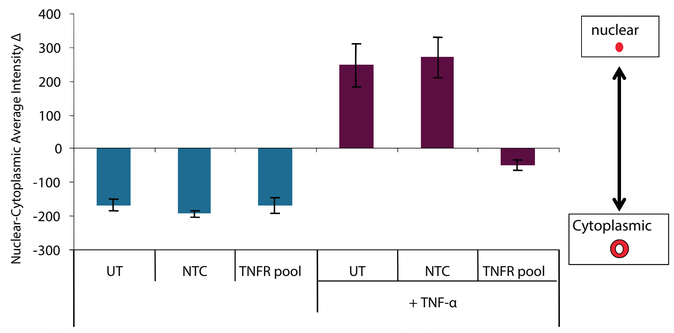
High-Content Analysis – Accell siRNA induces expected phenotype



NFkB translocates to nucleus upon activation by TNF-α

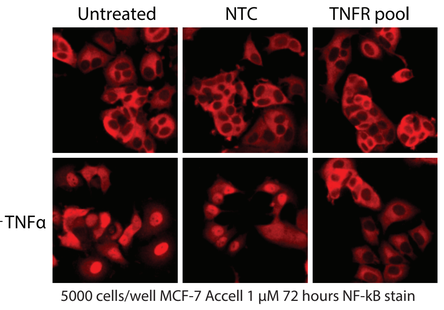
siRNA silencing the TNF-α receptor (TNFR) prevents translocation

Quantification of Accell NF-kB translocation phenotype in MCF-7 cells



MCF-7 5000 cells/well 72 hours Accell 1 µM TNF-α 10 ng/mL (30 minutes)

Accell siRNA targeting TNFRα prevents NF-kB translocation



5000 cells/well MCF-7 Accell 1 µM 72 hours NF-kB stain

Conclusions

Chemically modified siRNA that can be delivered to numerous difficult-to-transfect cell types without transfection reagents, viral vectors, or instrumentation was successfully developed

High confidence in experimental outcome
- Minimal non-specific delivery effects observed at the protein and the transcript level
- Expected phenotype observed for TNFα receptor Accell siRNA

Extended duration silencing
- Target silencing of up to 30 days can be obtained

Successful delivery into difficult-to-transfect cells such as T-cells and primary neurons

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