Efficient Delivery of siRNA using RJH Reagents

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About RJH Biosciences

We develop novel transfection reagents that deliver different types of nucleic acids to a range of mammalian cells in culture, while tailoring the transfection agents further to act as delivery vehicles for preclinical models and clinical therapy involving nucleic acids. Our reagents display exceptional activities on specific types of cells, while acting broadly for delivery of different types of nucleic acids.

Transfection Reagents

We offer specific and broadly acting transfection reagents to modify cells with DNA and RNA. The reagents are polymeric in nature and have been optimized for a variety of cell types and applications involving cell culture (*in vitro*) and animal models (*in vivo*). We offer reagents tailored for primary and suspension cells, as well as adherent cell lines.

Clinical Development

We are developing delivery systems to implement nucleic acid therapeutics in a clinical setting. Our goals are to realize the therapeutic potential of nucleic acid involved in RNAi (siRNA) and transgene expression (pDNA and mRNA). Partnerships are actively sought for various preclinical and clinical programs.

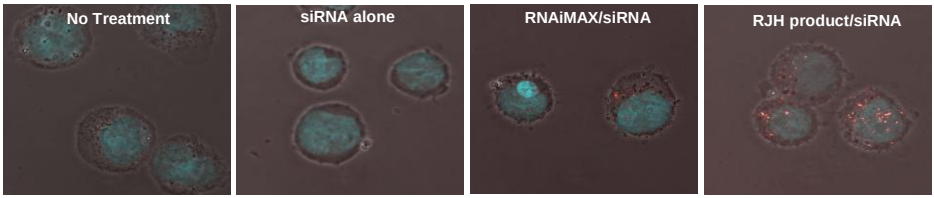
R&D Services

We offer high quality research and development services associated with transfection optimization and construct validation. Our goal is to provide the best delivery materials for your cargo and cell of choice. Our services are assessed and initiated by a quote request via the screening services page on our website or by a simple email.

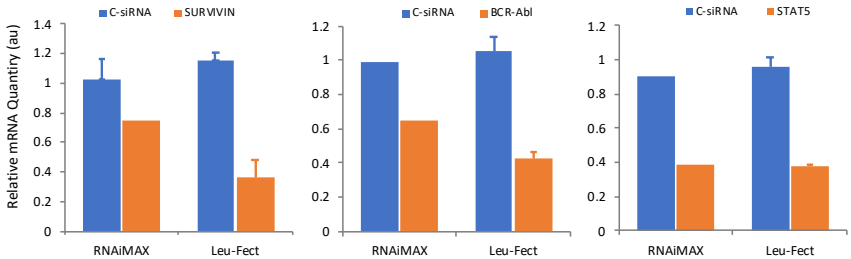
Delivery of siRNA *in vitro*

At RJH Biosciences, we have extensive experience working with siRNA., We offer several reagents that can be used to deliver siRNA to various cell types, with data to back it up! Whether it's a siRNA library screening assay or for a functional outcome, our reagents can provide effective delivery.

Gene Silencing using siRNA in Leukemia Cell Lines

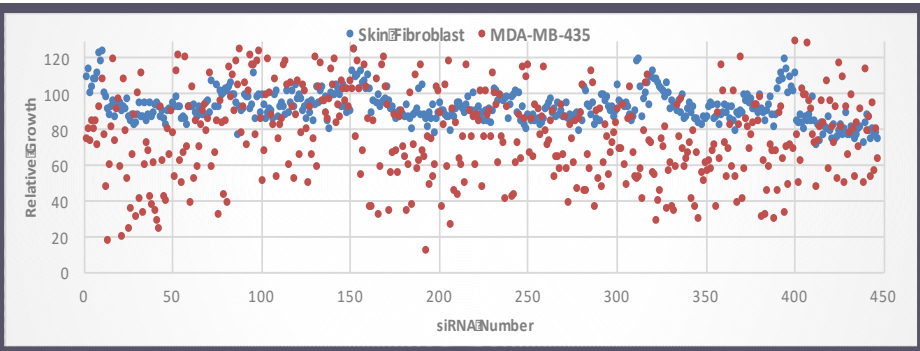


Uptake of siRNA complexes in K562 cell line as determined by confocal microscopy. Cells were exposed to either no transfection reagent (siRNA alone), RNAiMAX reagent (Invitrogen) or a RJH Biosciences reagent complexed with siRNA (as shown by the red fluorescence).



Gene silencing in leukemic K562 cells. Control (scrambled) siRNA and gene specific (survivin, Bcr-Abl and STAT5) siRNAs were transfected using Lipofectamine™ RNAiMAX (Invitrogen), or a RJH transfection reagent. The mRNA levels were determined by qPCR.

Delivery of an siRNA Library for Inhibiting Cell Growth



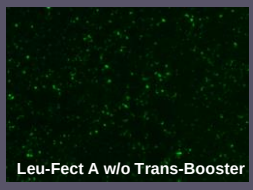
siRNA library screening in human breast cancer MDA-MB-436 cells and human foreskin fibroblast cells using a transfection reagent from RJH Biosciences. The relative growth of the two cell types was determined after treatment (3 days) with a library of apoptosis-related siRNAs. The growth was normalized against non-treated cells (taken as 100%).

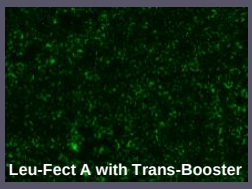
siRNA Transfection in Neuronal Cells

Delivery of siRNA into neural cells can be extremely difficult. At RJH Biosciences, we developed several reagents that provide high transfection efficiencies in neuronal cells with siRNA and other nucleic acids. Our line of products also include Trans-Booster, which can improve transfection efficiencies even further!

Delivery of siRNA into Neuro 2a Cells with RJH Reagents

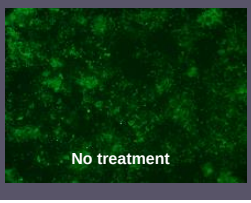
FAM-labeled siRNA delivery to Neuro 2a cells using Leu-Fect A and with/without Trans-Booster. Successful uptake using Leu-Fect A is observed with the addition of Trans-Booster greatly boosting transfection efficiencies. Transfection was determined using microscopy.

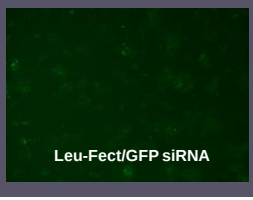




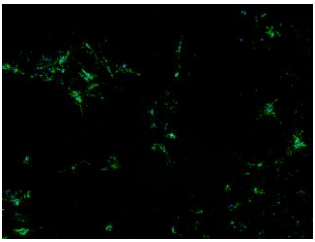
Silencing of a Reporter Gene (GFP) with siRNA in Neuro 2a Cells

Silencing of GFP-tagged mutant HTT (Exon 1) protein in Neuro 2a cells. Cells were transfected using Leu-Fect reagent and Trans-Booster complexed with either a GFP siRNA and a control siRNA**.** GFP expression is efficiently silenced in cells with minimal toxicity.





Delivery of siRNA into primary neurons (DRGs)

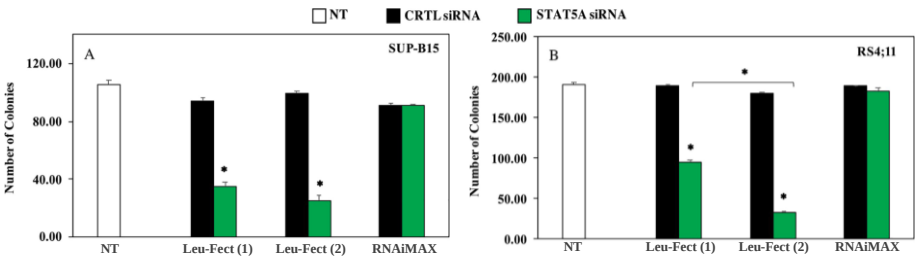


FAM-siRNA transfection into primary neurons (DRGs, dorsal root ganglion) using Leu-Fect A and Trans-Booster. DRGs were isolated from Sprague-Dawley rats. High siRNA uptake is observed in most cells (merged fluorescence image showing green Fam-labeled siRNA and blue/DAPI stained cell nucleus) as determined by fluorescent microscopy.

Delivery in Hard-to-Transfect Cells

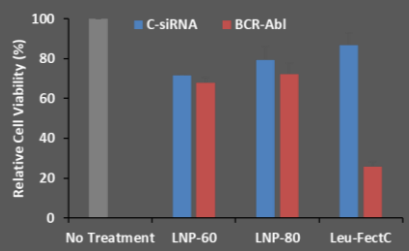
One challenge with the transfection of nucleic acids is finding the right materials to deliver into hard-to-transfect cell types. RJH Bioscience reagents are well tailored to provide delivery in hard-to-transfect cells such as primary cells, immune cells, and suspension-growing cells.

Delivery of siRNA into Leukemia Cells

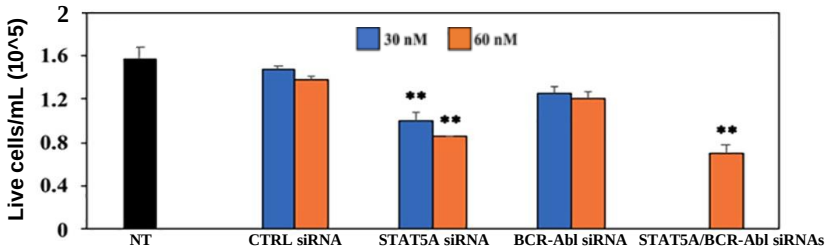


Growth inhibition of leukemic cell lines transfected with control and STAT5A siRNA complexes. Colony counts in agarose gels were performed two weeks after the treatment of cells at 60 nM siRNA concentration and at RJH reagent:siRNA ratio of 6, (RNAiMAX:siRNA ratio: 2.5:1 as recommended by the manufacturer). (A) SUP-B15 cells and (B) RS4;11 cells (n = 3). * p<0.05. Data from DOI: journal.pone.0251719(1).

Inhibition of Leukemic Cell Proliferation after siRNA Treatment



Effect of BCR-Abl siRNA treatment on proliferation of K562 cells. Cells were treated with siRNA concentration of 60 and 80 nM formulated with the clinical Lipid Nanoparticle Formulation (LNP) DLin-MC3-DMA and with 60 nM Leu-Fect C formulation (RJH Biosciences) at Leu-Fect C:siRNA ratio of 6:1. Cell viability was determined by trypan blue exclusion assay after 3 days.

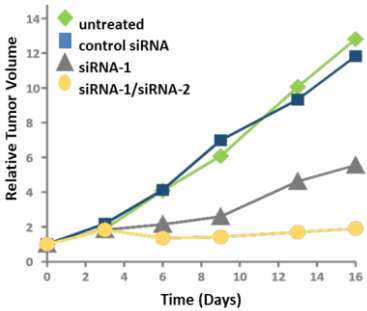


Effect of siRNA treatment on proliferation of BCR-Abl(+) patient cells. Cells were treated for 3 days with 30 and 60 nM siRNA with transfection reagent:siRNA ratio of 6:1. The siRNA used were control, STAT5A, and BCR-ABL specific siRNAs. Live cells were counted by trypan blue exclusion assay (n=3); ** P<0.01. Data from DOI: 10.1371/journal.pone.0251719

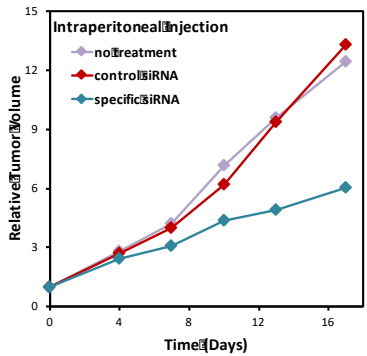
Delivery of siRNA in Animal Models

The RJH reagents go beyond the *in vitro* applications. They can effectively deliver nucleic acids such as siRNA into animal models as well, facilitating the clinical translation of siRNA therapies. Let us support your preclinical models by providing the best transfection efficiencies possible.

Inhibition of Tumor Growth with siRNA Injections

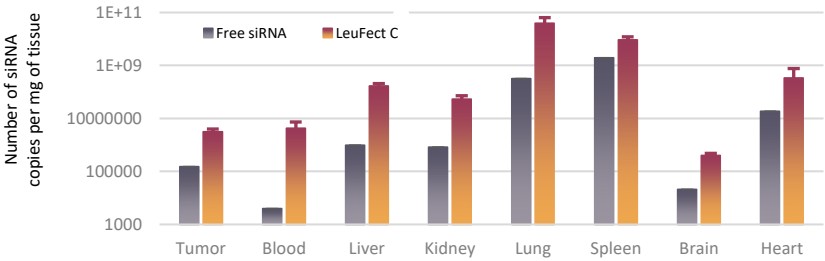


Inhibition of breast cancer tumors with local siRNA injections. Subcutaneous MDA-MB-231 tumors were established in nu/nu mouse. siRNAs were formulated with the Prime-Fect RJH transfection reagent and injected (x3) subcutaneously. Tumor growth was assessed over a period of 16 days by measuring the relative tumor volume. Relative tumor volume was obtained by measuring the tumors by external calipers and normalizing the volumes to initial tumor volumes (taken as 1).



Inhibition of breast cancer tumors with systemic siRNA injections. Intrapraperitonal injections were performed after subcutaneous breast cancer MDA-MB-231 tumor were established in nu/nu mouse. siRNAs (a control scrambled siRNA and a specific siRNA) were formulated with a the Prime-Fect RJH transfection reagent for injection. Tumor growth was assessed over a period of 16 days. Relative tumor volume was obtained by measuring the volumes by external calipers and normalizing the volumes to tumor initial volumes (taken as 1).

Biodistribution of siRNA in an Animal Model (NCG mouse)

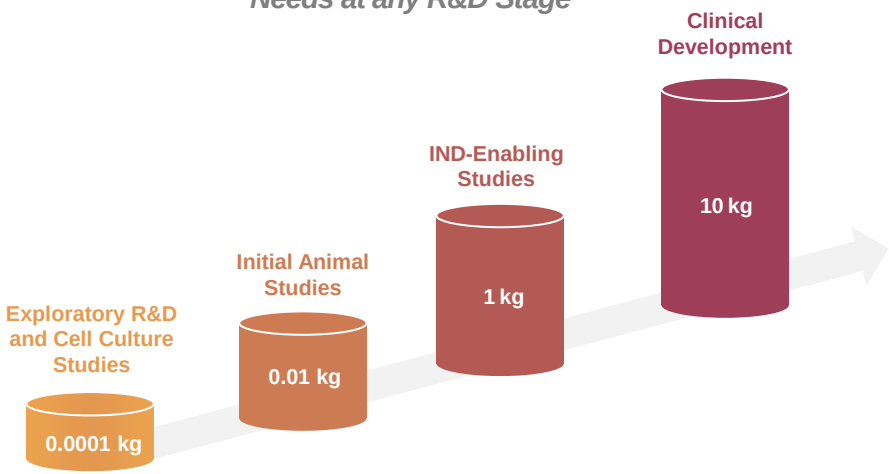


Biodistribution of siRNA. Leu-Fect reagent was used to deliver a specific siRNA by intravenous injection. Organ levels of the injected siRNA was determined with a ddPCR after 24 hr. The siRNA levels were normalized with the mass of the tissue recovered.

Upscaling siRNA Delivery and Applications

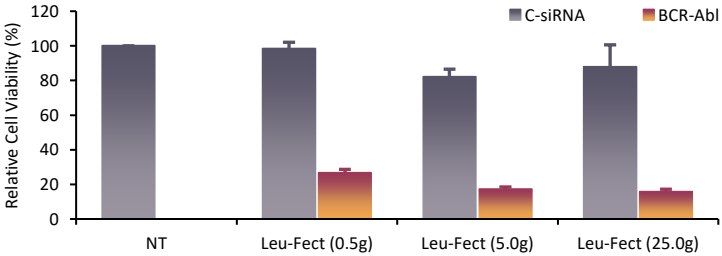
Access to large amounts of transfection reagents for preclinical and clinical studies is imperative for commercial success. RJH Biosciences prepares its reagents at a wide range of volumes without the loss of performance. We can provide materials over a wide range of scales.

RJH Biosciences can Service your Transfection Needs at any R&D Stage



For each stage of the development pathway, a certain amount of transfection reagent is typically needed. The exact amounts will depend on the efficacy (or effective dose) of the nucleic acid formulation with the delivery agent. An increasingly higher amount of materials need to be available whose performance is reproducible at different manufacturing levels. At RJH Biosciences, we can upscale the preparation of transfection reagents at levels that support general research (GxP) and pre-clinical and IND-enabling studies (GLP). Capabilities for clinical studies (GMP) are under development and we look forward to working with partners on clinical development activities.

Performance of Leu-Fect Prepared at Different Scales



Testing the efficacy Leu-Fect reagent produced at different scales. Leu-Fect reagent from 0.5, 5 and 25 g synthesis scales was used to deliver BCR-Abl siRNA to K562 cells. Leu-Fect successfully transfected the cells and induced equivalent cell killing in all cases.

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