Delivery of Nucleic Acids in Animal Models 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.

Product Selection

We have a variety of reagents suitable for delivery of nucleic acids to animal models. The table below summarizes the recommended use of RJH reagents for various nucleic acids. The RJH products have been found to be effective with an ever-expanding list of applications involving different nucleic acids in animal models. Please contact us for further guidance and testing in different systems.

Transfection Reagent	Nucleic Acid		
	Plasmid DNA	mRNA	siRNA/miRNA
ALL-Fect / ALL-Fect Kit	√		√
Prime-Fect	√		√
mRNA-Fect / mRNA-Fect Kit		√	
Trans-Booster*	√	√	√

* supplied as part of transfection kits

RJH Products Tailored for Animal Studies

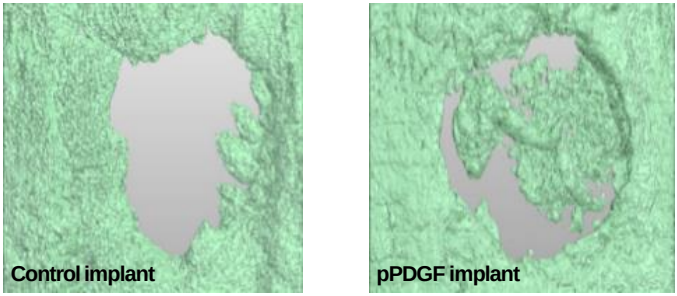
Product	Product No	Feature
ALL-Fect	10-10/20	delivery of small quantity of pDNA, siRNA
ALL-Fect In Vivo	10-30	delivery of large quantity of pDNA, siRNA
ALL-Fect Kit	10-40/50/60	with Trans-Booster for improved transfection
Prime-Fect	20-10/20	delivery of small quantity of pDNA, siRNA
Prime-Fect In Vivo Kit	20-40/50	delivery of large quantity of pDNA, siRNA with Trans-Booster for improved transfection
mRNA-Fect	80-10/20	delivery of small quantity of mRNA
mRNA-Fect In Vivo	80-30	delivery of large quantity of mRNA
mRNA-Fect Kit	80-40/50/60	with Trans-Booster for improved transfection

ALL-Fect for *In Vivo* Applications

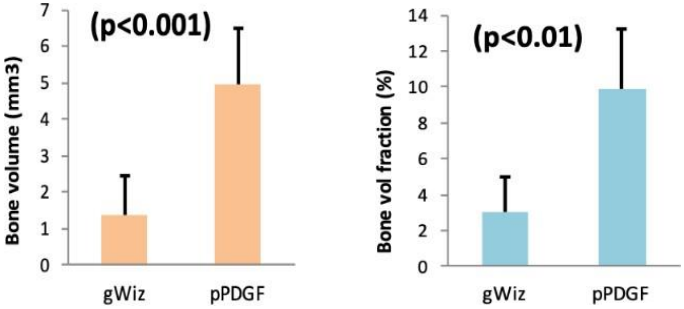
ALL-Fect is a highly effective, broadly-acting transfection reagent for delivery of pDNA and siRNA in animal models. It can be used for delivery of individual nucleic acids as well as combinations of different nucleic acids.

Repair of Bone Tissue in a Rat Skull Defect with Implantation of pDNA

µCT Images of Typical Bone Defects after 4 Weeks



Histomorphometric Parameters from µCT Analysis (n=5)

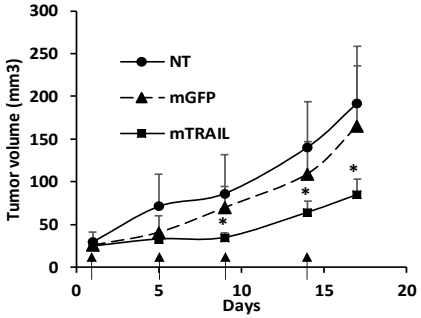


pDNA gene delivery in a rat calvarial defect model to stimulate bone tissue regeneration. 4 mm defects were created in SD Rats (n=5/group) and implanted with Gelfoam™ sponges containing a non-expressing control pDNA (gWIZ) or PDGF expressing pDNA, both condensed with **ALL-Fect** transfection reagent. MicroCT images for representative individual rats are shown on the top panel while the quantitative analysis of the defects on the bottom panel (analysis at 4 weeks post-implantation). The unrepaired areas devoid of bone tissue are indicated with gray regions in the microCT images. Among the several morphological parameters analyzed, bone volume and bone volume fraction (as representative parameters) were significantly higher for the pPDGF treated defects compared to control treated defects.

mRNA-Fect for *In Vivo* Applications

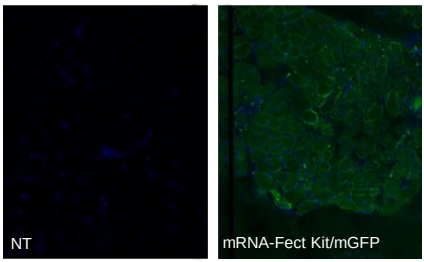
The mRNA-Fect reagent is a highly effective transfection reagent optimized for mRNA delivery in various animal models. Along with Trans-Booster as part of a kit, mRNA-Fect can deliver a mRNA payload to circulating as well as attached cells in an *in vivo* setting.

Inhibition of Tumor Growth with Local mRNA injections



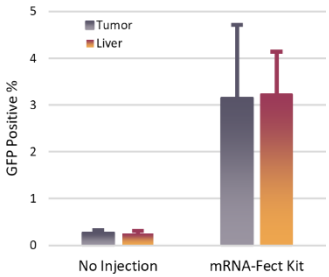
Growth of breast cancer SUM149 xeno-grafts. Study groups corresponding to No treatment (NT), mRNA coding for GFP (mGFP) and TRAIL (mTRAIL) are shown. Data represent mean±SD of 5-8 animals at each time. mRNA was formulated with **mRNA-Fect**, and injected (lower arrows) in the vicinity of tumors. Tumor volume after treatment of mTRAIL complexes was significantly less after day 9 (* p<0.05).

GFP Expression in Mouse Tibialis Anterior Muscle



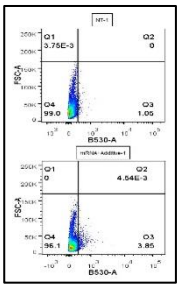
GFP expression in tibialis anterior muscles of NCG mice.
NT (No-treatment): Injection with RPMI.
mRNA-Fect Kit/mGFP: mGFP injection with **mRNA-Fect Kit**.
The animals were analyzed after 2 days of injection for GFP expression. There was no GFP expression in the NT group. A robust GFP expression was evident in the animals treated with mGFP complexed using the **mRNA-Fect Kit**.

Systemic Delivery of mRNA

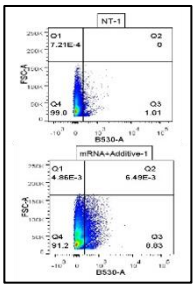


GFP mRNA delivery to mice using mRNA-Fect Kit. Transfection was quantified using flow cytometry to measure the percentage of GFP(+) cells in SC tumour and liver tissues 48 hrs after IV injection. mRNA-Fect Kit enables high efficiency mRNA delivery *in vivo*.

Tumor Analysis



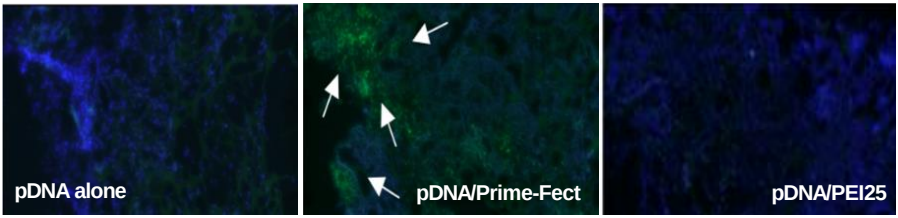
Liver Analysis



Prime-Fect for *In Vivo* Applications

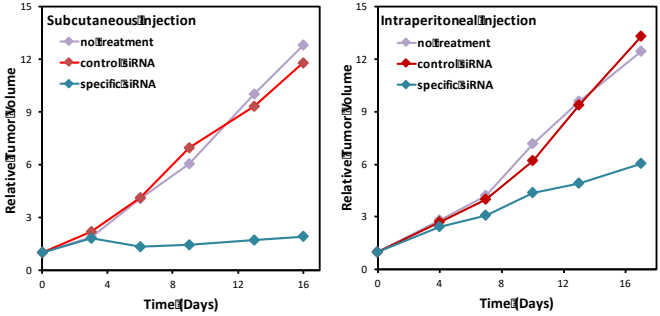
Prime-Fect is a highly effective transfection reagent optimized for pDNA and siRNA delivery in various animal models. It is particularly suitable for delivery of nucleic acids to tissues constituted with attachment dependent cells.

Expression of Reporter GFP Gene in Implants with pDNA



Delivery of plasmid DNA (pDNA) using Prime-Fect. A GFP expression plasmid (AcGFP) was used for subcutaneous implantation in Sprague-Dawley rats with collagen implants. The implants contained pDNA alone, or pDNA complexed with **Prime-Fect** (pDNA/**Prime-Fect**) or 25 kDa branched PEI (pDNA/PEI25). The implants were recovered after 14 days of in-life, and visualized for GFP expression by epifluorescent microscopy. GFP positive cells were primarily observed when **Prime-Fect** was used for plasmid delivery (see arrows).

Inhibition of Tumor Growth with siRNA Injections

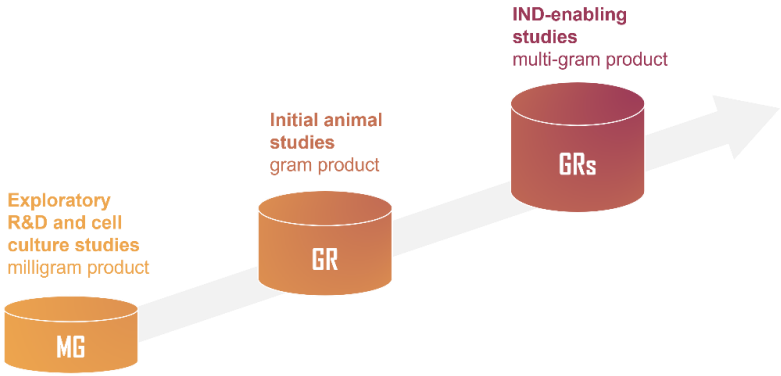


Tumor growth profiles after siRNA delivery with Prime-Fect. (Left) Subcutaneous breast cancer MDA-MB-231 tumors were established in nu/nu mouse. siRNAs (a control scrambled siRNA and a specific siRNA) formulated with **Prime-Fect** were injected (x3) subcutaneously and tumor growth was assessed over a period of 16 days. (Right) Subcutaneous tumors from MDA-MB-231 cells were established in nu/nu mouse. siRNAs (a control scrambled siRNA and a specific siRNA) formulated with **Prime-Fect** were injected (x3) intraperitoneally and tumor growth was assessed over a period of 16 days. Relative tumor volume was obtained by measuring the tumor volumes by external calipers and normalizing the measured volumes to initial volumes.

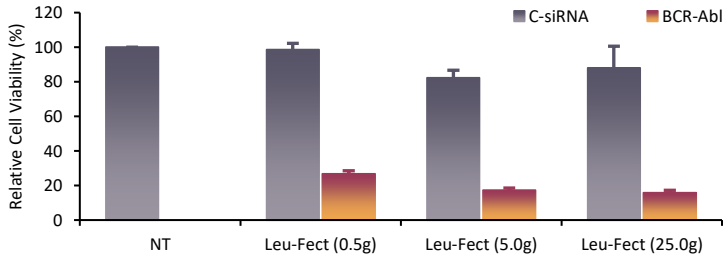
Producing **RJH Reagents** at Large Scale

RJH reagents provide high efficiency nucleic acid delivery to a wide array of cell types as well as in animal models. The process to prepare the reagents are readily scaled-up to prepare reagents with reproducible performance over a wide range of scales.

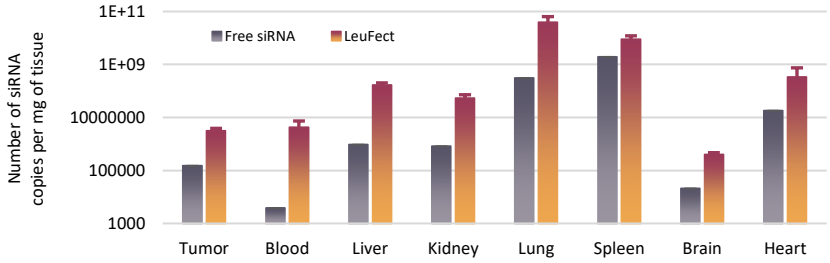
RJH Biosciences can service your transfection needs at any scale of production



Performance of Leu-Fect Prepared at Different Scales



Testing the efficacy Leu-Fect reagent produced at different scales. Leu-Fect reagent from 500 mg, 5 g and 25 g 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.



Biodistribution of siRNA. Leu-Fect reagent from 25g scale was used to deliver a specific siRNA by intravenous injection. Organ levels of the siRNA was determined with a ddPCR.

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