RJH BIOSCIENCES

RJH Reagents are Safe for Animal Model Applications

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About RJH Biosciences

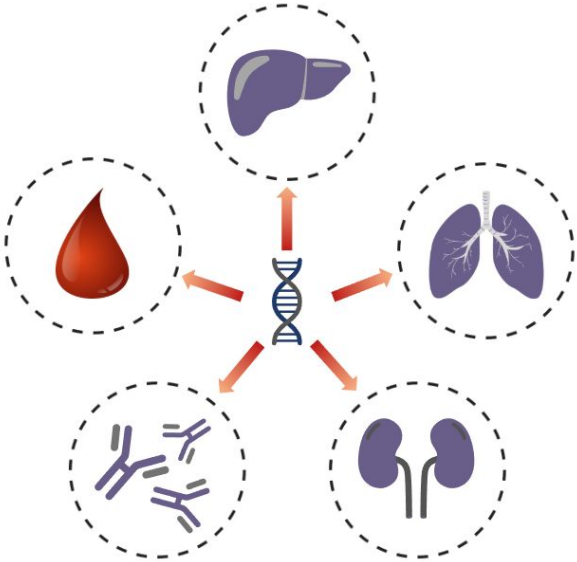
We develop novel transfection reagents that deliver different types of nucleic acids to a range of mammalian cells in culture, while tailoring the transfection agents further to act as delivery vehicles for preclinical models and clinical therapy involving nucleic acids. Our reagents display exceptional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

Transfection Reagents

We offer 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 are proud to offer transfection reagents tailored for primary cells and suspension cells, as well as adherent cell lines and animal models.

Clinical Development

We are developing novel nucleic acid delivery systems to effectively implement nucleic acid therapeutics in a clinical setting. Our goals are to realize the potential of RNA interference (RNAi) via delivery of siRNA, and enabling transgene expression via direct administration of plasmid DNA (pDNA) and mRNA to express proteins in situ. Partners are actively sought for various preclinical and clinical programs.



Introduction to Biosafety

Polymer-based nucleic acid therapeutics use RNA or DNA molecules to regulate gene expression, treat diseases, and deliver genetic instructions with enhanced safety and reduced toxicity. These therapies, such as small interfering RNA (siRNA) and messenger RNA (mRNA), rely on polymers to improve stability, control release, and facilitate cellular uptake. By targeting specific genes, these systems enable precise treatments while minimizing off-target effects. Efforts focus on optimizing polymers to reduce toxicity and enhance biosafety, ensuring effective therapies with minimal side effects. Nucleic acid-based therapeutics including vaccines, like those developed for COVID-19, highlight the importance of developing safe delivery systems for a rapid response to various health challenges.

Delivery

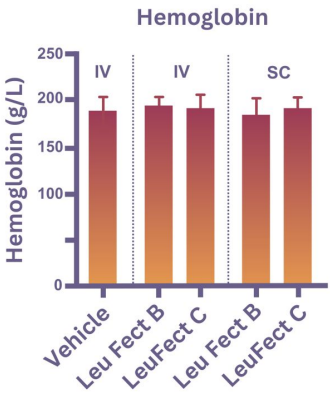
Delivering nucleic acid therapeutics using polymer-based carriers presents challenges related to potential toxicity, as certain polymer properties—such as charge, size, or composition—can trigger immune responses or harm healthy cells. However, our team has dedicated extensive efforts to ensuring that our polymers are safer and more biocompatible. Through rigorous testing and refinement, we focus on selecting non-toxic materials and fine-tuning polymer structures to minimize cytotoxicity. This continuous optimization ensures that our polymers effectively deliver nucleic acids with a reduced risk of adverse effects, enhancing therapeutic delivery while prioritizing safety.

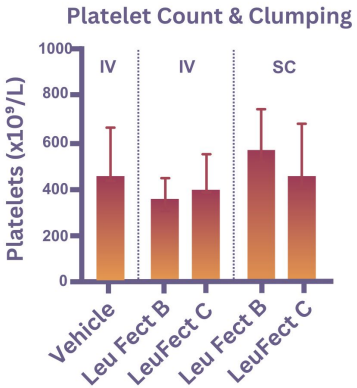
Blood Chemistry

Leu-Fect is a versatile delivery reagent capable of efficiently delivering siRNA to various *in vivo* xenograft models, ensuring effective biodistribution and target gene silencing across multiple tissues.

Leu-Fect exhibits effective and safe biodistribution of siRNA in mice, achieving high siRNA delivery across multiple tissues in in vivo models while maintaining biosafety.

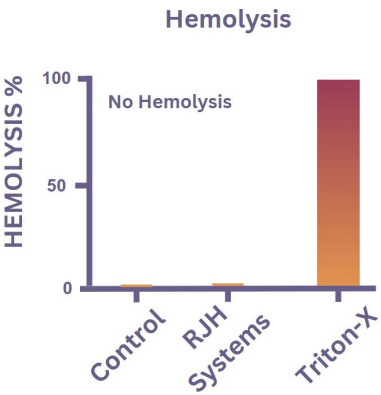
Hemoglobin levels in mice following IV and SC delivery of Leu-Fect B and C. Hemoglobin concentrations (g/L) were measured and showed no significant differences between control and treatment groups, indicating the safety of Leu-Fect formulations.





Platelet counts in mice following IV and SC delivery of Leu-Fect B and C. Platelet levels were measured and showed no significant differences between control and treatment groups, further confirming the safety of Leu-Fect formulations.

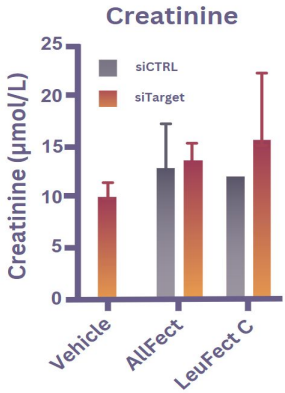
Hemolysis assessment confirmed the safety of various RJH pDNA delivery systems. Averaged across all polymers, RJH systems showed no hemolysis, comparable to the control, while Triton X-100 induced 100% hemolysis. This underscores the exceptional biosafety of RJH formulations for pDNA delivery.



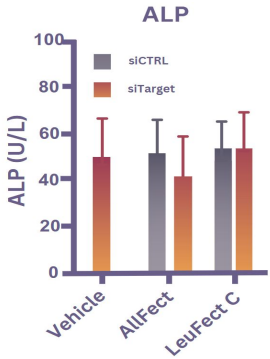
Organ Function and Toxicity

Organ function and toxicity analyses show minimal impact, supporting the biosafety of RJH reagents.

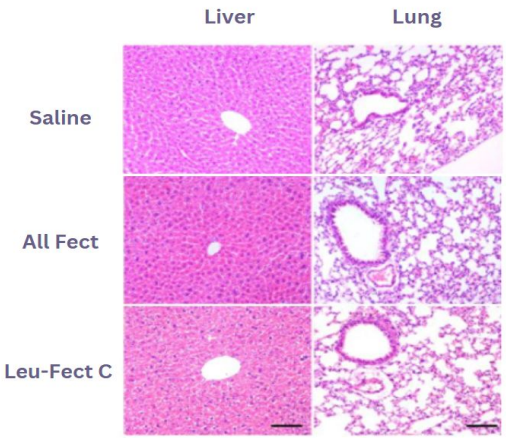
Histopathology, enzyme activity, and cytokine analyses confirm low toxicity and minimal immune response, highlighting the safety of RJH reagents.



Creatinine levels in mice following injection of control or target siRNA formulations with All Fect or Leu Fect C. No significant differences in creatinine levels were observed between the treatment groups and the control, suggesting that the polymers may not significantly affect kidney function.



ALP levels in mice following injection of control or target siRNA formulations with All Fect or Leu Fect C polymers. No significant differences in ALP levels were observed between the treatment groups and the control, indicating that the polymers may not significantly affect liver function.



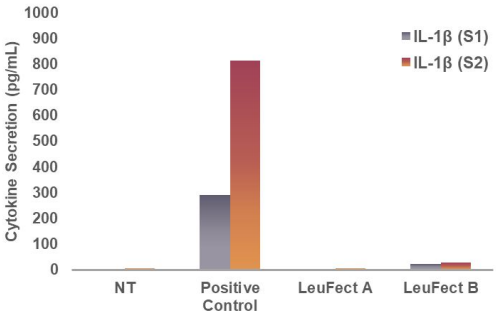
Histopathological analysis of liver and lung. Tissue sections showed no significant differences between saline, All Fect, and Leu Fect C mRNA treatments, indicating that both polymers are safe for systemic delivery with no apparent organ toxicity. The scale bar represents 100 µm.

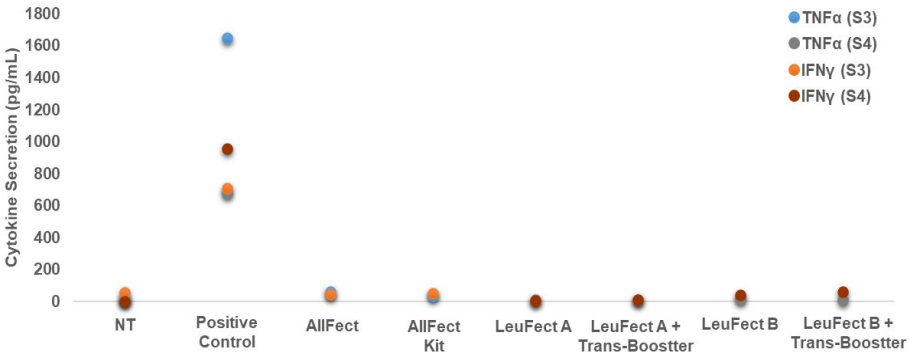
Cytokine Immune Stimulation

RJH reagents exhibit excellent biosafety by inducing no detectable cytokine secretion, ensuring minimal immune activation during treatment.

Cytokine analysis confirms low IL-1beta, TNF-alpha and IFN-gamma levels across all formulations, highlighting their compatibility with immune system parameters.

IL-1β Cytokine levels after treatment. No IL-1beta cytokine stimulation was observed in two different human PBMCs (S1 and S2) treated with RJH reagents carrying siRNA, suggesting no immune response. Significant cytokine release was seen in the positive control group, indicating a strong immune activation.





TNFα and IFNγ cytokine quantitation. TNF-alpha and IFN-gamma cytokine levels in two different PBMC samples (S3 and S4) were significantly lower with RJH reagents carrying pDNA compared to the controls, indicating minimal immune activation across a wide range of formulations.

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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 siRNA and mRNA delivery. 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.

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
Leu-Fect A	30-10/20	delivery of large quantity of siRNA, microRNA, and ASO
Leu-Fect B	40-10/20	delivery of large quantity of siRNA, microRNA, and ASO
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

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