

# Research & Development Services on Transfection Technologies

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## About R&JH 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.

# Transfection Reagent Screening Services

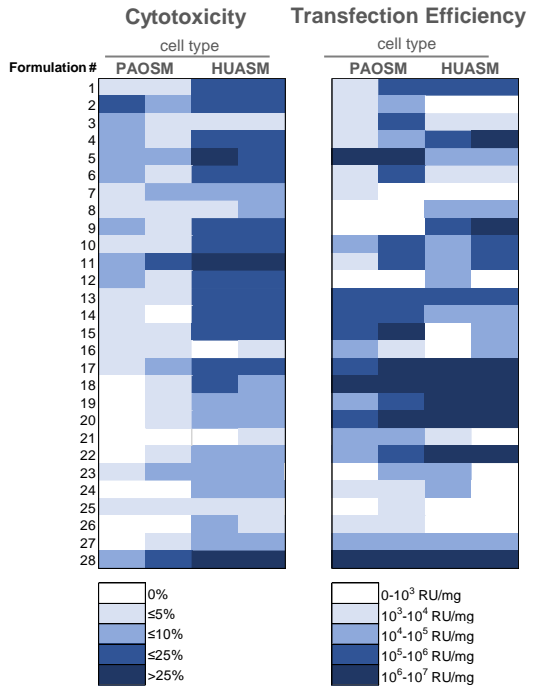
The first step to finding the best transfection reagent often comes with screening leading products. At RJH Biosciences, we compare within our own materials at different polymer:nucleic acid ratios and with other commercial reagents to achieve the best possible transfection efficiencies.

## Screening for siRNA Delivery



**Screening for siRNA delivery reagents in 5 separate breast cancer cell types with a transfection reagent library (RJH Biosciences).** The heat map shows the effectiveness of different polymer formulations for siRNA delivery in MCF-7, MDA-MB-231, MDA-231K, AU565, and MDA-486 cells. Uptake into cells was assessed by FAM-labeled siRNA and categorized into 4 separate groups, based on the percentage of FAM-siRNA positive cell populations in the treatment groups.

## Screening for pDNA Delivery

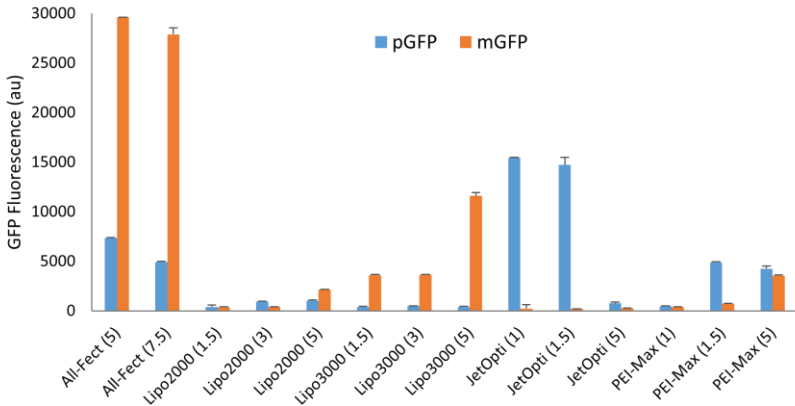


**Cytotoxicity and transfection efficiency of a transfection reagent library (RJH Biosciences) in smooth muscle cells.** The cell types were porcine aortic smooth muscle cells (PAOSM) and human umbilical artery smooth muscle cells (HUASM). The cells were transfected with pGL3 plasmid at 5 and 10 w/w reagent:pDNA ratios. Transfection was assessed based on relative luminescence after 48 hours. Cytotoxicity was determined by AlamarBlue assay at the same time. Data is represented as a heat map according to induced luminescence (RU/mg of protein) and viability loss (%).

# Reagents for Easy- and Hard-to-Transfect Cell Models

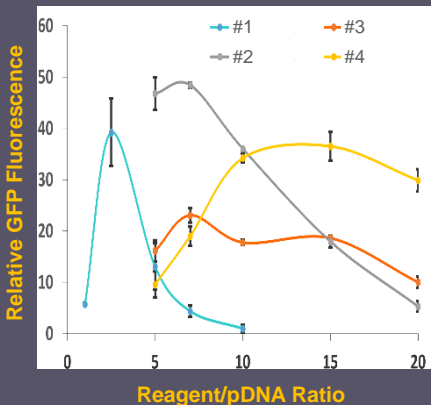
At RJH Biosciences, we tackle both easy-to-transfect and hard-to-transfect cells models, both attachment-dependent readily endocytosing cells as well as suspension-growing cells that resist uptake of foreign nucleic acids.

## pDNA and mRNA Delivery to Suspension-Growing Jurkat Cells



**Comparison of transfection efficiencies using RJH transfection reagents and commercially available materials.** pDNA and mRNA molecules expressing GFP reporter were transfected into Jurkat T-cells using either RJH reagent All-Fect, or other commercial reagents Lipofectamine™ 2000 and 3000, jetOPTIMUS, or PEI-MAX. Various transfection reagent:DNA ratios (w/w) were used to optimize each product. Extent of GFP transfection per cells was determined using flow cytometry 3 days post transfection and compared among the modified cells.

## Optimizing pDNA Delivery to Attachment-Dependent Cells

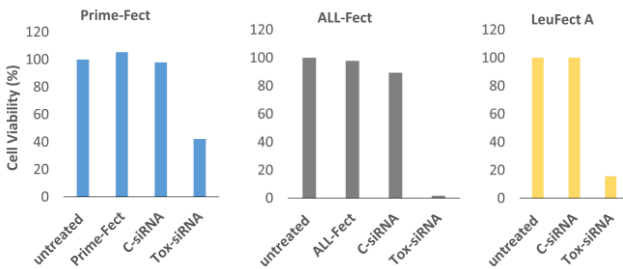


**Optimization of protein production after transfection with various reagents.** The 293T cells, a workhorse in biotech industry for protein production, were transfected with a GFP expressing plasmid using 4 different transfection reagents from RJH Biosciences. Different reagent:pDNA ratios (w/w) were used to identify the optimal ratio for transgene expression in the cells. Expression of GFP was determined via flow cytometry. Data is represented as relative GFP fluorescence.

# Validation Assays: siRNA Activity

Beyond the transfection, it is important to assess the functional activity of the delivered DNA, RNA, or CRISPR RNPs. RJH Biosciences can conduct initial studies with functional assays, so that you can focus on the other aspects of your projects. Various experiments can be undertaken based on the specific application.

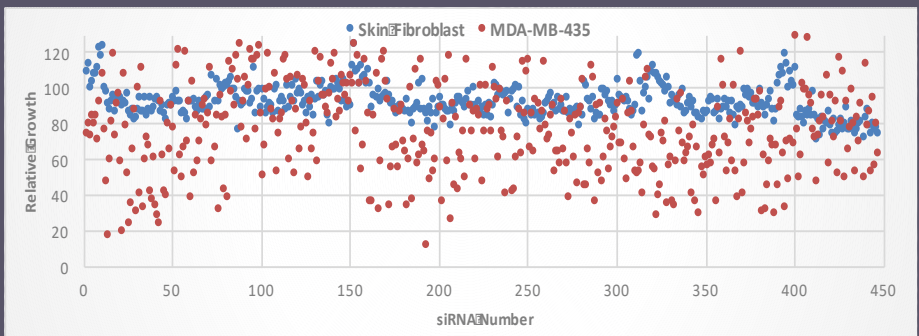
## siRNA Delivery for Cell Killing



### Cell killing with RJH reagents.

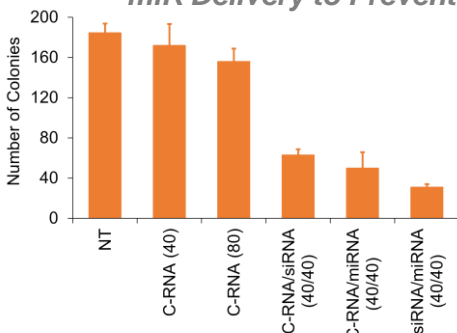
The study groups were untreated cells, cells treated with the RJH reagents alone, cells treated with reagent delivered scrambled siRNA (C-siRNA) and cytotoxic siRNA (Tox-siRNA). Results are summarized as %cell viability.

## Delivering 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 apoptotic siRNAs. The growth was normalized against non-treated cells (taken as 100%).

## miR Delivery to Prevent Leukemic Colony Formation

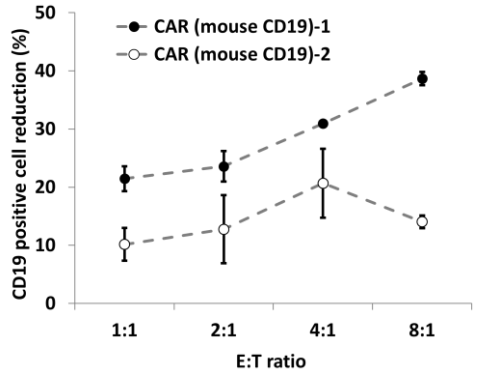


**Colony Forming Unit (CFU) assay in K562-IMR cells using killing siRNA/miRNA.** Cells were treated with a control RNA (C-RNA), a specific siRNA against BCR-ABL oncogene, and a proprietary miRNA (40 nM, 80 nM, or 40+40 nM). Cells were transfected with LeuFect A (RJH Biosciences) complexed with the nucleic acids for 24 hours and then transferred to methylcellulose gels for further cultivation. The number of CFUs from the modified cells were counted at the end of 2-week period (in triplicate).

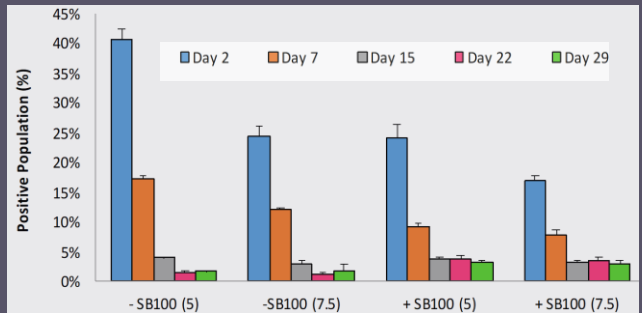
# Validation Assays: Immunotherapy

For the development of advanced treatments such as immunotherapy, assessment at the preclinical level is essential. Along with providing the best transfection reagents possible, we can also validate your DNA/RNA technology in an application-based setting. Experiments can be tailored and optimized based on your applications.

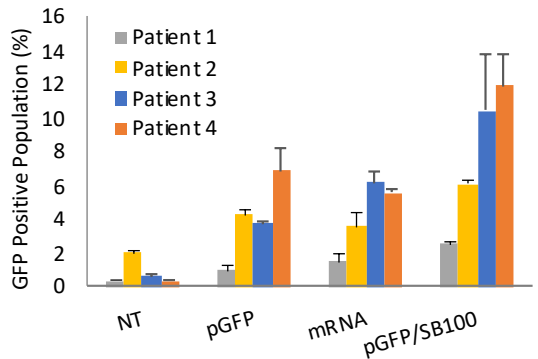
**Cell killing assay with co-culturing of effector (CAR-modified Jurkat) and target (mouse WEHI) cells.** Reduction in the percentage of CD19(+) WEHI cells when co-cultured with Jurkat T-cells (modified with one of 2 different pDNAs coding for anti-hCD19 CARs) was determined at different effector:target (E:T) ratios. The percentage of remaining cells was accessed 3 days after initiation of co-culture by flow cytometry and CD19 antibody staining.



**Longevity of transfection in T-cells.** A SB transposon system (SB100) that includes expressible GFP was transfected into Jurkat T-cells using the ALL-Fect transfection reagent (RJH Biosciences) at different transfection reagent:pDNA ratios (in brackets). Percentage of cells expressing GFP was determined by flow cytometry at several time points over the span of 29 days.



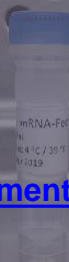
**Peripheral Blood Mononuclear Cell (PBMC) Transfections.** Transfection efficiency was determined using PBMCs (whole blood) from four sources. Variance among the donor PBMCs was determined with no transfection group (NT) or transfection of pDNA encoding a GFP SB transposon, a GFP expressing mRNA, and a complete GFP SB transposon system (transposon donor and transposase coded by a pDNA mixture). The pDNA/mRNA was delivered using the ALL-Fect reagent. GFP expression was determined by flow cytometry 2 days post transfection.



# Start your quote today!

Fill out a form at

<https://rjhbiosciences.com/researchanddevelopmentservice/> or email us at [info@rjhbiosciences.com](mailto:info@rjhbiosciences.com)



## Benefits of using our R&D Services

- Affordability
- Savings from time consuming and laborious screening and optimization protocols involving transfections
- Improved delivery of nucleic acids to specific cell types, ensuring optimal activity assessment
- Reliable and consistent nucleic acid delivery, reducing experiment to experiment variations
- Identification of unique reagents optimized for your cell type.

## Benefits of using our Reagents

- **Multivalent interactions** with nucleic acids leading to strong binding of cargo that withstand disruptive forces in transit through cell membranes.
- **pH buffering capacity** that facilitate escape of cargo from endosomes.
- **Lipidic moieties** that enhance interactions with cell membrane and internalization.
- **Synergistic effects** that coat the cargo and protect it from nucleases.
- **Tailored formulations** to free nucleic acids once internalized in cytoplasm.
- **Aqueous buffers** that obviate exposure of cells to organic solvents.

### Terms of services:

The information given to RJH Biosciences through the forms or otherwise will be kept confidential unless the customer agrees to data release that will be used for promotional purposes (such as the testimonial or application notes on the RJH website) only. Services will only commence after confirmation by the customer and RJH Biosciences (discussions to verify the optimal screening process will occur prior to confirmation). RJH Biosciences has a typical service turnaround time of 30 days, which may vary depending on project requirements and success. RJH Biosciences does not guarantee that the services will be successful. If unsuccessful, a monetary refund equivalent to 30% of the project costs will be given to the customer.

### Disclaimer:

RJH Biosciences is not liable for experimental limitations after the screening services has been finished and data has been collected, although we can provide additional resources and consultation if said issues arise. RJH Biosciences cannot guarantee the success of the proposed services. RJH Biosciences cannot guarantee the success of the project service if used in other cell lines and primary cell lines that are different from the originally tested. RJH Biosciences also cannot guarantee the success of the project in different models.



**RJH Biosciences Inc.**

Suite 4000, 10230 Jasper Avenue,  
Edmonton, Alberta,  
T5J 4P6 CANADA

[https://rjhbiosciences.com/screening-services/  
rjhbiosciences@gmail.com](https://rjhbiosciences.com/screening-services/rjhbiosciences@gmail.com)