

# Tailored Transfection Reagents for CRISPR/Cas9 Gene Editing

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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

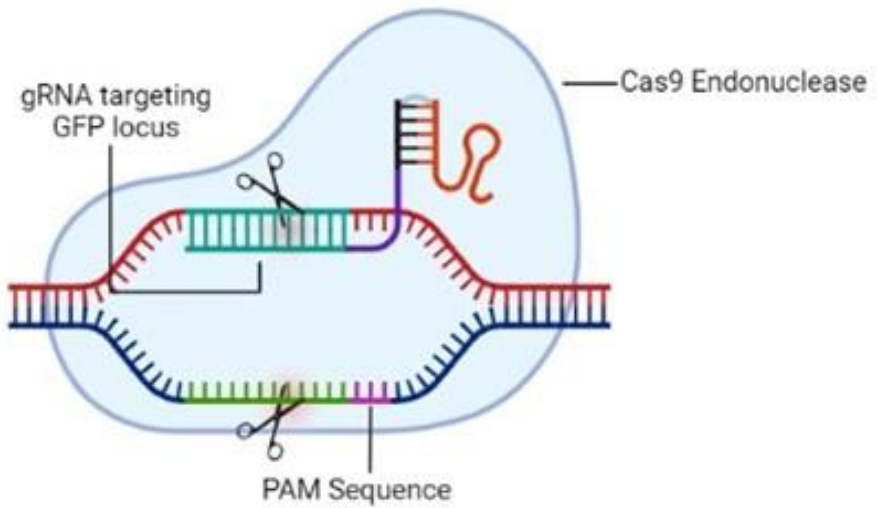
We offer specific and broadly acting transfection reagents to modify cells with DNA and RNA. The reagents are polymeric in nature and have been optimized for a variety of cell types and applications involving cell culture (*in vitro*) and animal models (*in vivo*). We offer reagents tailored for primary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



## What is CRISPR?

The CRISPR-Cas9 system can facilitate editing of any site in an organism's genome with high specificity. Recent developments have seen it utilized in plants, mammals, and other eukaryotic species and cell lines. The CRISPR-Cas9 system consists of two components: an endonuclease (the Cas9 protein) and a single guide RNA (sgRNA) that complexes with the Cas9. The complex targets a specific site in the genome via Watson-Crick base-pairing interactions between the sgRNA and genomic DNA, where the Cas9 cleaves the DNA. Host cell mechanisms then attempt to repair this break, which can result in "knocking-out" expression of the cleaved gene through insertion/deletion mutations or "knocking-in" of a transgene if a DNA vector template is provided.

## Delivery

CRISPR-Cas9 systems can be implemented by using a plasmid DNA or an mRNA that expressed the Cas9 protein *in situ*. The sgRNA can be similarly expressed, or supplied as an exogenous/synthetic agent. Alternatively, Cas9 can be formulated into a ribonucleoprotein (RNP) complex composed of the Cas9 protein and the synthetic sgRNA. RJH Biosciences has developed several chemical transfection reagents capable of delivering all three CRISPR-Cas9 systems, including RNP complex, whose delivery was limited by current transfection methods.

## Product Selection

We have a variety of reagents to enable Cas9-mediated gene modification with plasmid DNA and mRNA coded Cas9, we well ribonucleoprotein (RNP) bearing Cas9. The table below summarizes the suitability of the RJH reagents with various Cas9 complexes. The RJH products have been found to be effective with an ever-expanding list of nucleic acids and cell models. We also provide reagents for delivery of nucleic acids in animal models. Please contact us for trial packs for initial testing in different systems.

### Transfection Reagents for Various Methods of CRISPR

Transfection Reagent	CRISPR Approach		
	Plasmid Cas9	RNP Cas9	mRNA Cas9
All-Fect	✓	✓	✓
Prime-Fect	✓	✓	
CRISP-Fect		✓	✓
Trans-Booster*	✓	✓	

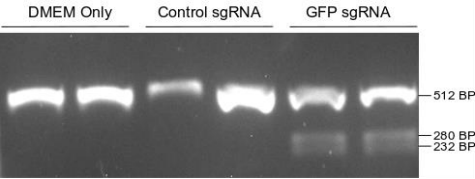
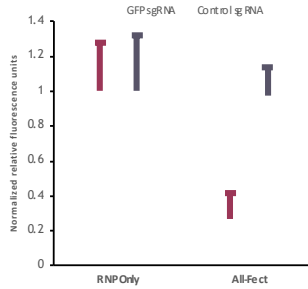
\*: supplied as part of transfection kits

# All-Fect for CRISPR Applications

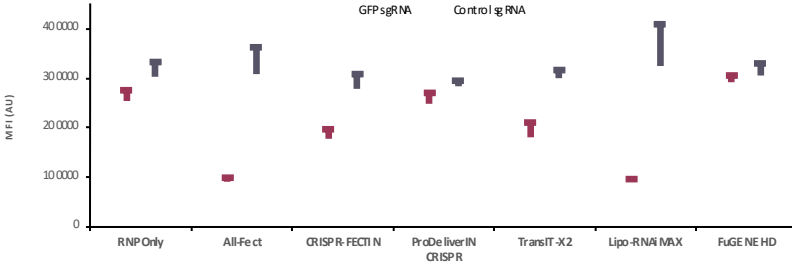
All-Fect is a broadly acting transfection reagent capable of delivering pDNA, siRNA, and Cas9/sgRNA RNP complexes to a variety of cell types, including suspension cells and primary cells.

All-Fect is effective for Cas9-mediated knock-out of GFP expression in breast cancer MDA-MB-231 cells and neuronal N2a cells using Cas9/sgRNA RNPs.

**Transfecting Neuro 2A cells with Cas9/sgRNA RNP complexes using All-Fect.** GFP expression levels were analyzed using fluorescence spectroscopy in multiwell plates. High efficiency GFP gene editing with no discernable toxicity was achieved using All-Fect.



**T7E1 Assay on GFP locus of GFP(+) MDA-MB-231 cells transfected using All-Fect.** Editing of the GFP locus by Cas9 are observed with a specific sgRNA, but not control sgRNA.



**Transfecting MDA-MB-231 cells with Cas9/sgRNA RNP complexes using All-Fect and other commercial reagents.** GFP expression levels were analyzed using flow cytometry and summarized as mean fluorescence/cell. All-Fect is highly efficacious compared to other reagents.

## All-Fect

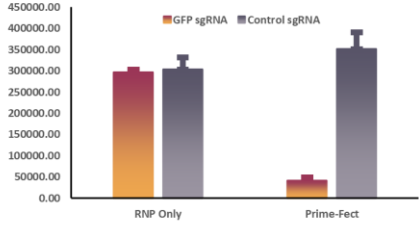
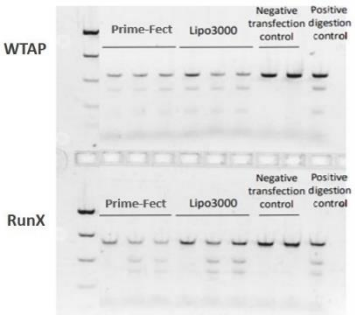
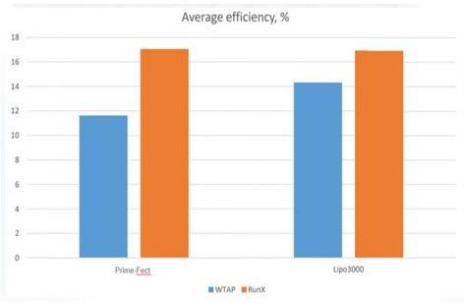
- SKU (Size)** #10-10 (0.75 mL), #10-20: (1.5 mL)
- Nucleic Acid** pDNA, siRNA, Cas9/sgRNA complexes
- Cell Types** Cell lines, stem, primary, suspension cells
- Serum** Compatible
- Buffer** Aqueous
- Storage Temp.** -20 °C
- Shelf Life** 1 year

# Prime-Fect for CRISPR Applications

Prime-Fect was designed for pDNA delivery in hard-to-transfect primary cells. It is capable of effective delivery of CRISPR-Cas9 plasmids and Cas9/sgRNA complexes to a variety of cell types.

Prime-Fect has been used to deliver CRISPR-Cas9 plasmids to kidney fibroblast 293-T cells and Cas9/sgRNA RNP complexes to breast cancer MDA-MB-231 cells.

**Transfecting kidney fibroblast 293-T cells with Prime-Fect.** CRISPR editing by Cas9 plasmids of WTAP and RunX has comparable efficiency with a leading lipofection reagent on the market (below). A T7 Endonuclease Assay of wild-type and edited genomic DNA clearly shows editing of the WTAP and RunX loci (right).



**Transfecting MDA-MB-231 with Cas9/sgRNA RNP complexes using Prime-Fect.** Cas9-mediated knock-out of GFP expression was analyzed using flow cytometry. Prime-Fect shows high efficiency GFP knock-out.

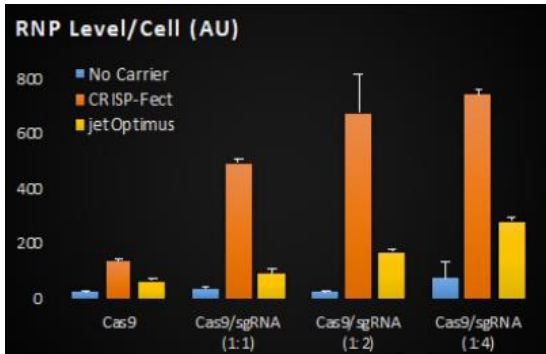
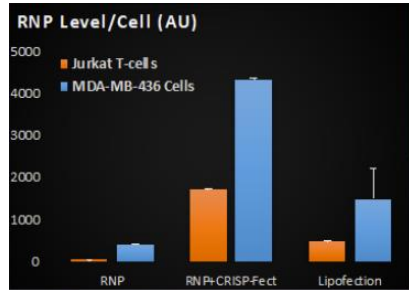
## Prime-Fect

<b>SKU (Size)</b>	#20-10 (0.75 mL), #20-20 (1.5 mL)
<b>Nucleic Acid</b>	pDNA, siRNA, Cas9/sgRNA complexes
<b>Cell Types</b>	Primary (pDNA), cell lines (siRNA)
<b>Serum</b>	Compatible
<b>Buffer</b>	Aqueous
<b>Storage Temp.</b>	-20 °C
<b>Shelf Life</b>	1 year

# CRISP-Fect for CRISPR Applications

CRISP-Fect is optimized for RNP delivery to both attachment-dependent and suspension-growing cells. CRISP-Fect has shown high efficiency delivery of Cas9 RNPs to a variety of cell types.

**Transfection of Jurkat T-cells and breast cancer MDA-MB-436 cells using CRISP-Fect with Cas9/sgRNA complexes.** RNP uptake was analyzed by using FITC-labeled Cas9 (courtesy of CasZyme Corp.) and measuring fluorescence. In both cell types, CRISP-Fect was highly efficient in delivering RNPs to cells, compared to a commonly used lipofection reagent.



**Optimization of Cas9/sgRNA ratios in Jurkat T-cells for RNP Uptake.** CRISP-Fect has consistently provided higher transfection efficiencies than a competing commercial reagent.

## CRISP-Fect

<b>SKU (Size)</b>	#90-10 (0.75 mL), #90-20 (1.5 mL)
<b>Nucleic Acid</b>	Ribonucleoprotein
<b>Cell Types</b>	Suspension, attachment-dependent cells
<b>Serum</b>	Compatible
<b>Buffer</b>	Aqueous
<b>Storage Temp.</b>	-20 °C
<b>Shelf Life</b>	1 year



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