tailored transfection reagent: CRISP-Fect

PRODUCT NUMBERS: 90-10 and 90-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: -20 °C

The **CRISP-Fect** reagent is a highly effective transfection reagent optimized for ribonucleoprotein (RNP) delivery to both attachment-dependent and suspension-growing cells. **CRISP-Fect** is able to bind RNPs complexes of Cas9 endonuclease and sgRNA. Upon co-incubation, the transfection reagent forms 50-150 nm particles with RNPs that bear a net positive charge. The formation of complexes is performed in aqueous buffers without any organic solvents during preparation. **CRISP-Fect** is a non-integrating reagent and does not cause genetic changes on its own after treatment. **CRISP-Fect** has been confirmed to provide effective delivery of Cas9 RNPs in different cell types as noted on our Transfection Reagent selection guide. Figure 1 shows typical delivery of RNPs to attachment-dependent cells (breast cancer cells) and suspension-growing cells (Jurkat T-cells). As with all transfection reagents, formulations of **CRISP-Fect** with the intended cargo may need to be optimized for specific cell types and transfection conditions.

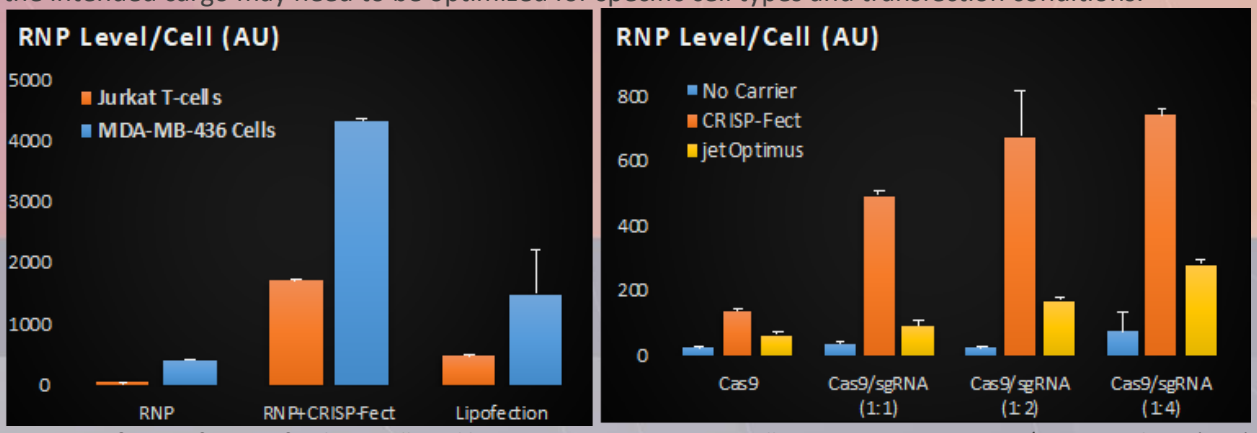

Figure 1. Left. Transfection of Jurkat T-cells and breast cancer MDA-MB-436 cells using CRISP-Fect. sgRNA/Cas9 complexes (RNP) were formulated with indicated transfection reagents and added to the cells for 24 hours. RNP uptake was followed by using FITC-labeled Cas9. The results are summarized as arbitrary fluorescence units, indicating the average amount of RNPs delivered per cell. **Right.** Transfection of Jurkat T-cells with RNPs formulated at different ratios of Cas9/sgRNA using CRISP-Fect. Lipofectamine 2000 and jetOptimus was used as commercial reference reagents in these studies.

| Benefits of CRISP-Fect

- High transfection efficiency in the presence of serum.
- Effective delivery of pDNA or RNP's via a simple protocol that is ideal for scale-up and automation.
- Minimal toxicity compared to other commercial reagents, minimally affecting highly sensitive human cells.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.
- Possibility of using the same transfection reagent in animal models, leading to harmonized studies.
- Cost-effective reagent minimizing additional costs in sgRNA screens (with Cas9).

| Notes on Transfection Protocol

The following procedure is recommended for preparation of RNP complexes with **CRISP-Fect** and subsequent transfection of cells. Please ensure all reagents are at room temperature for the procedures.

- We recommend using freshly passaged cells at exponential growth phase for transfection.

- Cells can be seeded at desired concentrations in multiwell plates before addition of complexes (normal transfection). If cells are attachment-dependent, allow 24 hours for cells to attach and spread. For suspension-growing cells, complexes could be incubated in multiwell plates first, followed by the addition of desired numbers of cells (reverse transfection).
- Recommended amounts of RNP and **CRISP-Fect** reagent are shown in Table 1. Recommendations for RNP formation is a molar ratio between 1:1 and 1:4 for Cas9:sgRNA and a final Cas9 concentrations of 0.25-1.0 µg/mL. We recommend a final concentration of less than 2 to 5 µg/mL for **CRISP-Fect**, with typical RNP: **CRISP-Fect** ratio of 1:5. We recommend all concentrations and reagent ratios to be optimized for specific cell types. The amounts shown below are for a single well, assuming 0.1 mg/mL RNP and 1 mg/mL **CRISP-Fect** solutions. Complex volumes should be adjusted based on the number of replicates.
- We recommend preparation of 10% excess volume to account for any possible loss due to pipetting.
- DMEM (or equivalent) cell culture medium without antibiotics or serum is recommended for complex preparation but the medium can be changed depending on the need of the cells.

Table 1: Recommended reaction components of RNP: **CRISP-Fect** for transfection

Plate Format	Medium (µL)	RNP (µL)*	CRISP-Fect (µL)	Medium Volume (µL) per well
96-well	10	0.5	0.25	90
48-well	30	1.5	0.75	270
24-well	60	3.0	1.5	540
6-well	300	15	7.5	2700

* Recommended volumes for 0.1 mg/mL RNP and 1 mg/mL **CRISP-Fect** solutions (RNP: **CRISP-Fect** ratio is 1:5).

| Step-by-Step Procedure

1. Add desired volume of medium to 1.5 mL plastic (microcentrifuge) tubes.
2. Mix sgRNA and Cas9 at a molar ratio of 2:1 and incubate at room temperature for 30 min.
3. After incubation, add appropriate volume of RNP solution to the medium in tubes and vortex for 3 sec.
4. Add undiluted **CRISP-Fect** solution to RNP solution. Gently vortex for 3 sec and incubate for 20-30 min.
5. Re-suspend the complexes in solution using a pipette at the end of the incubation period.
6. For normal transfection, add complexes to wells containing the attached cells that were seeded the day before in complete medium. Gently shake plates if necessary to allow even distribution of complexes.
7. For reverse transfection, add complexes to empty wells, followed by the addition of cells suspended in complete medium with serum. Gently shake the plate to ensure uniform distribution of cells in wells.
8. Incubate the plate under conditions suitable for cell culture (typically at 37 °C in a humidified atmosphere with 5% CO_2/95% air) and sample cells at desired times for analysis. We recommend 48 hours for analysis.