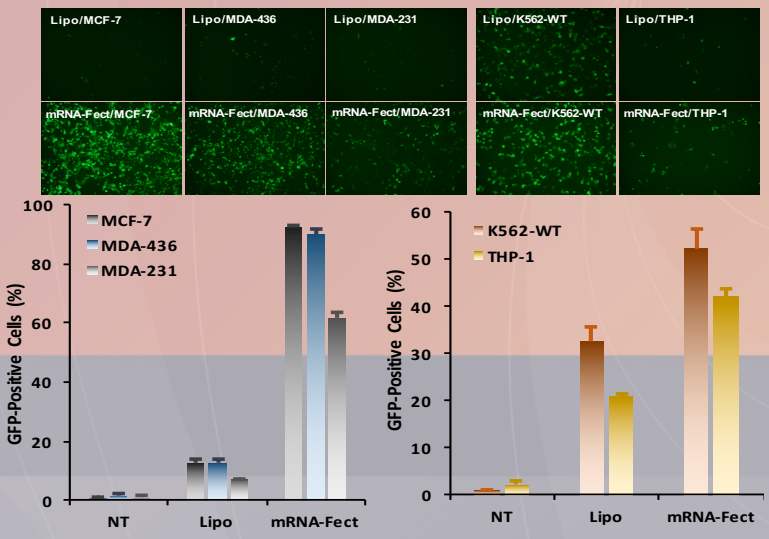
Tailored Transfection Reagent: *mRNA-Fect*

PRODUCT NUMBERS: 80-10 and 80-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: -20 °C

Product Description

The **mRNA-Fect** reagent is a highly effective transfection reagent optimized for mRNA delivery to both attachment-dependent and suspension-growing cells. **mRNA-Fect** reagent is a synthetic cationic lipopolymer that is tailored primarily for mRNA delivery after extensive testing of amphiphilic polymer libraries. It is capable of undergoing multivalent interactions with polynucleotides and encapsulating co-incubated mRNA into polyionic nanoparticle of size 100 to 200 nm. The complexation between mRNA and **mRNA-Fect** occurs in aqueous buffers, obviating the need for organic solvents during preparation. The **mRNA-Fect** is a non-integrating carrier, so that the genetic make-up of host cells is not altered after the treatment. The **mRNA-Fect** has been tested and found effective for plasmid DNA and siRNA delivery to a wide range of cell types. As with all transfection reagents, formulations of **mRNA-Fect** with polynucleotides may need to be optimized for specific cell types and transfection conditions.

Transfecting various cells with mRNA-Fect, including attachment-dependent MCF-7, MDA-MB-436 and MDA-MB-231 cells, and suspension-growing K562 and THP-1 cells. An mRNA coding for a reporter protein (Green Fluorescent Protein, GFP) was used to assess the efficiency of mRNA expression. Typical GFP expression levels were visualized under fluorescent microscopy (top pictures). The expression levels were quantitated by flow cytometry 72 hours after transfection and summarized as the percentage of cells positive for GFP. For comparison, a leading lipofection reagent was used according to the manufacturer's instructions.



| Benefits of mRNA-Fect

- High transfection efficiency in the presence of serum.
- Effective delivery of mRNAs via a simple protocol that is ideal for scale-up and automation.
- Minimal toxicity compared to other commercial reagents, suitable for highly sensitive human cells.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.
- It is possible to use the same transfection reagent in animal models, leading to harmonized studies.
- Cost-effective reagent minimizing additional costs in mRNA screens due to transfection reagent

| Notes on Transfection Protocol

The following procedure is recommended for preparation of mRNA particles with **mRNA-Fect**, and subsequent transfection of attachment-dependent and suspension-growing cells. Please ensure all reagents are at room temperature for the procedures.

- Cells can be seeded at desired concentrations in multiwell plates. We recommend using freshly passaged cells at an exponential growth phase for transfection.
- If cells are attachment-dependent, allow 24 hours for cells to attach and spread and proceed transfection (forward transfection). For suspension-growing cells, complexes can be added in multiwell plates first, followed by the addition of desired numbers of cells (reverse transfection).

Plate Format	Medium (µL)	mRNA (µL) *	mRNA-Fect (µL)	Total Complexes (µL)	Culture Volume (µL)
96-well	8.625	0.5	0.375	10.0	90.0
48-well	15.87	1.5	1.125	20.0	280.0
24-well	41.75	3.0	2.25	50.0	550.0
6-well	258.75	15.0	11.25	300.0	2700.0

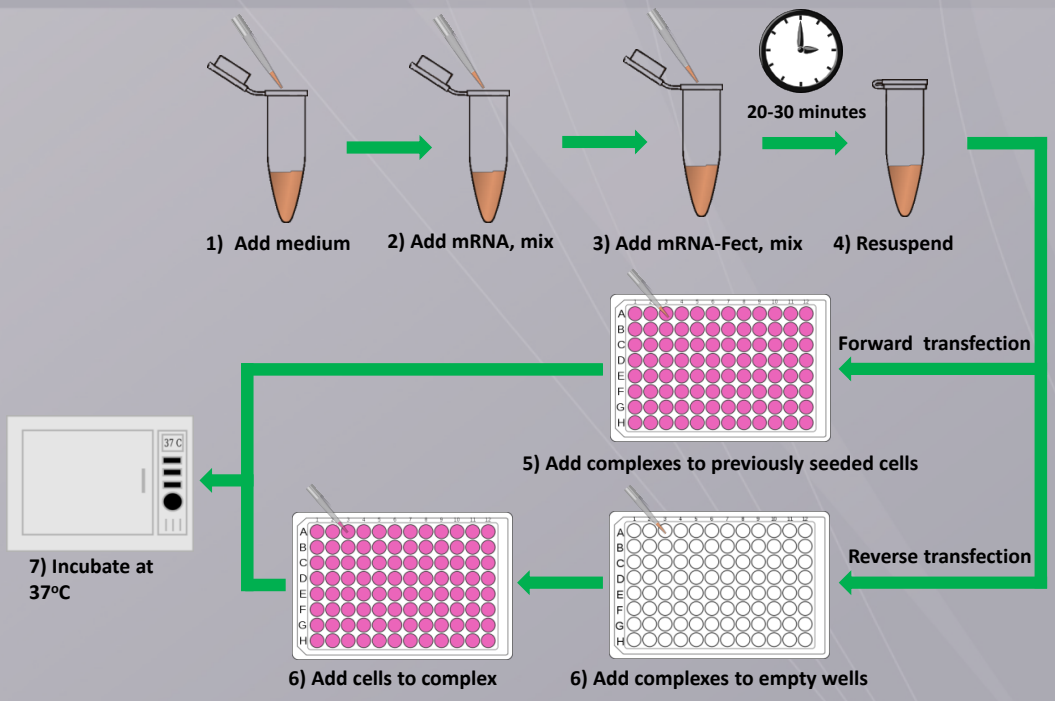
* Recommended volumes for forward transfection in a single well, assuming 0.1 mg/mL mRNA and 1 mg/mL **mRNA-Fect** solutions (mRNA: **mRNA-Fect** ratio is 1:7.5). Once the plate format is selected, complex volumes should be adjusted based on no of replicates.

- Recommended amounts of mRNA and **mRNA-Fect** are shown in the Table below for different multiwell plates. The final recommendations for mRNA conc are 0.25-1.0 µg/mL. We recommend a final concentration of 1.25 to 5 µg/mL for **mRNA-Fect**, with typical nucleic acid: **mRNA-Fect** ratio of 1:7.5. We recommend all concentrations and reagent ratios to be optimized for each cell type.
- We recommend preparation of 10% excess volume to account for any pipetting losses.
- 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.

| Step-by-Step Procedure

1. Add desired volume of medium to 1.5 mL plastic (microcentrifuge) tubes.
2. Add appropriate volume of mRNA solution to the medium in tubes and vortex gently for 3 sec.
3. Add undiluted **mRNA-Fect** solution to nucleic acid solution. Vortex for 3 sec and incubate for 20-30 min.
4. Re-suspend the complexes in solution using a pipette at the end of the initial incubation period.
5. **Forward transfection**: add complexes directly to the wells containing the previously seeded cells (allowed to attach for 24 hours in complete medium with serum). Ensure even distribution, gently shake plates if necessary.
6. **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.
7. Incubate the plate under cell culture conditions for culture. Sample cells at desired times for analysis.

| Graphical Procedure for transfection



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