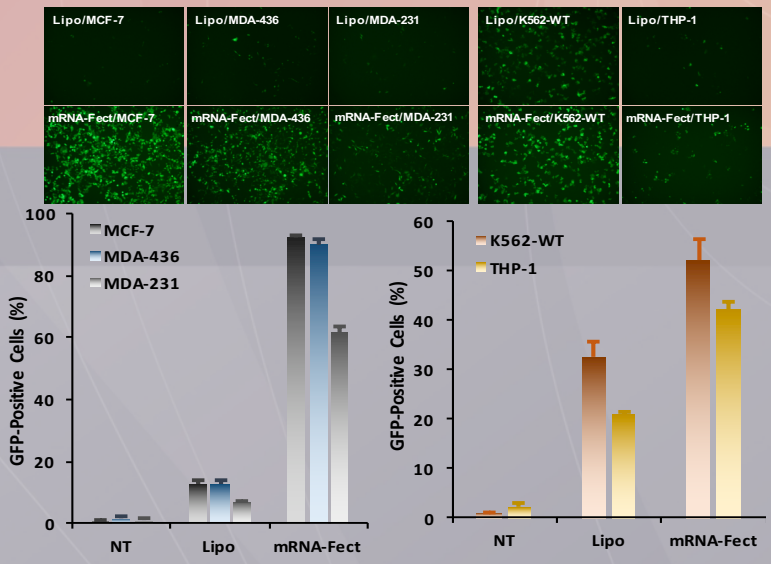
tailored transfection reagent: mRNA-Fect

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

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 amphiphilic polymer 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 approximately 100 nm particles with a net positive charge. The complexation between the mRNA and the **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 treatment with this reagent. The **mRNA-Fect** has been tested and found effective for plasmid DNA and siRNA delivery as well in certain 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 mRNA molecules 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 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.

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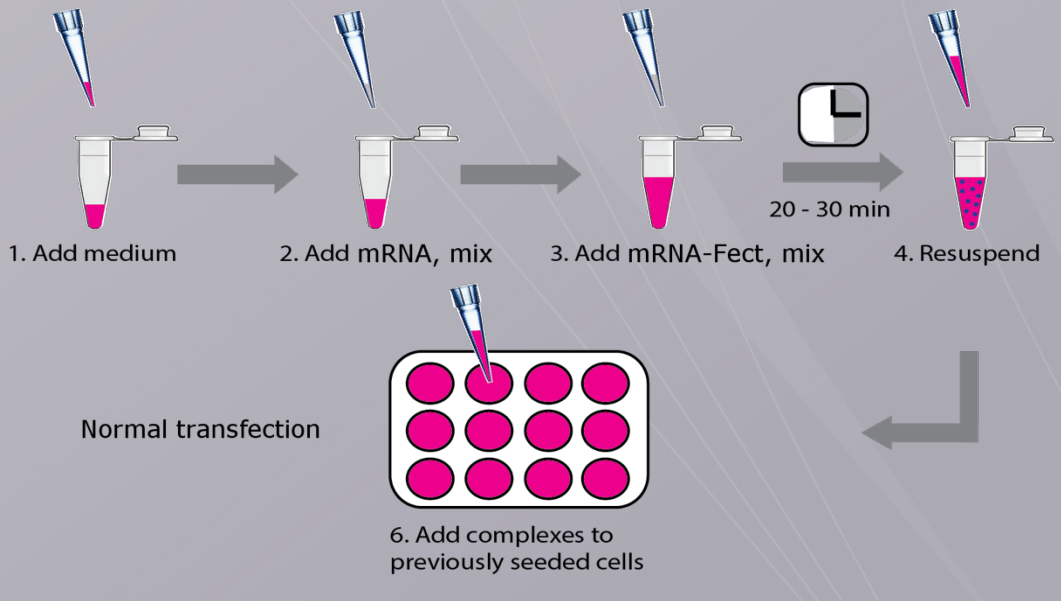
- We recommend to use freshly passaged cells at exponential growth phase for transfection.
- Cells can be seeded at desired concentrations in multiwell plates before addition of complexes (normal transfection). If cells are attachment-dependent, allow 24 hours for cells to attach and spread. For suspension-growing cells, complexes could be incubated in multiwell plates first, followed by the addition of desired numbers of cells (reverse transfection).
- Recommended amounts of mRNA and **mRNA-Fect** reagent are shown in the Table below for different multiwell plates. The final recommendations for mRNA are 0.25-1.0 µg/mL. We recommend a final concentration of less than 5 µg/mL for **mRNA-Fect**, with typical nucleic acid: **mRNA-Fect** ratio of 1:5. We recommend all concentrations and reagent ratios to be optimized in the hands of practitioners. The amounts shown below are for a single well, assuming 0.1 mg/mL mRNA and 1 mg/mL **mRNA-Fect** solutions. Once the plate format is selected, complex volumes should be adjusted based on no 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.

Plate Format	Medium (µL)	mRNA (µL)*	mRNA-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 mRNA and 1 mg/mL **mRNA-Fect** solutions (mRNA: **mRNA-Fect** ratio is 1:5).

| 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. For normal transfection, add complexes to 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. 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.
7. Incubate the plate under conditions suitable for cell culture (typically at 37 ^{o}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.



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