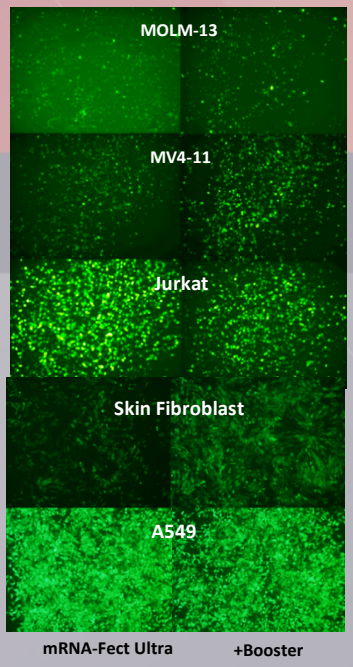
Tailored Transfection Reagent: *mRNA-Fect ULTRA*

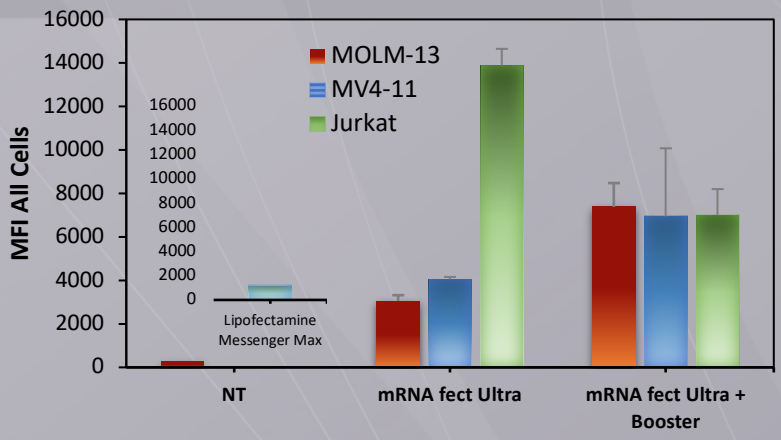
PRODUCT NUMBERS: 80-12 and 80-22	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: -20 °C

Product Description

mRNA-Fect ULTRA reagent is a cutting-edge solution specifically designed for efficient and reliable mRNA delivery to a wide variety of cell types, including both adherent and suspension cells. This advanced reagent is a synthetic cationic lipopolymer developed through extensive screening and optimization of amphiphilic polymer libraries, ensuring superior performance for mRNA applications. The reagent facilitates formation of stable nanoparticles, with sizes ranging between 100 to 200 nm, by forming robust interactions with mRNA molecules. The encapsulation occurs entirely in aqueous buffers, eliminating the need for organic solvents and simplifying the preparation process. The nanoparticles protect mRNA during delivery, enhancing stability and ensuring effective transfection. Unlike integrating carriers, this reagent does not alter the host cell's genetic material, making it an ideal choice for applications where maintaining the native genomic structure is critical. While primarily optimized for mRNA, its adaptability allows users to tailor formulations for specific cell types and experimental conditions, further expanding its versatility. Through rigorous testing, this reagent has demonstrated remarkable transfection efficiency, offering researchers a reliable tool to unlock the full potential of mRNA-based experiments and applications. Whether your focus is on gene expression studies, vaccine development, or therapeutic mRNA delivery, this transfection reagent sets a new standard in mRNA delivery technology.



Testing was conducted in suspension-growing Jurkat, MV4-11, and MOLM-13 cells, and attachment-dependent skin fibroblasts and A549 cells. An mRNA encoding the Green Fluorescent Protein (GFP) was utilized to assess transfection efficiency. GFP expression was visualized using fluorescent microscopy. For suspension-growing cells, quantitative analysis of GFP expression was performed by flow cytometry 48 hours post-transfection, summarizing the mean fluorescence intensity (MFI) of the cells. For comparison, a leading lipofection reagent was used in Jurkat cells following the manufacturer's protocol.



| Benefits of mRNA-Fect ULTRA

- 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 ULTRA**, 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 attachment before 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).
- Recommended amounts of mRNA and **mRNA-Fect ULTRA** 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 ULTRA**, with typical nucleic acid:**mRNA-Fect ULTRA** ratio of 1:7.5. We recommend all concentrations and reagent ratios to be optimized for each cell type.

Plate Format	Medium (µL)	mRNA (µL) *	mRNA-Fect ULTRA (µL)	Total Complexes (µL)	Culture Volume (µL)
96-well	9.1	0.5	0.4	10	90
48-well	17.3	1.5	1.2	20	280
24-well	44.6	3.0	2.4	50	553
6-well	253	15.0	12.0	280	2715

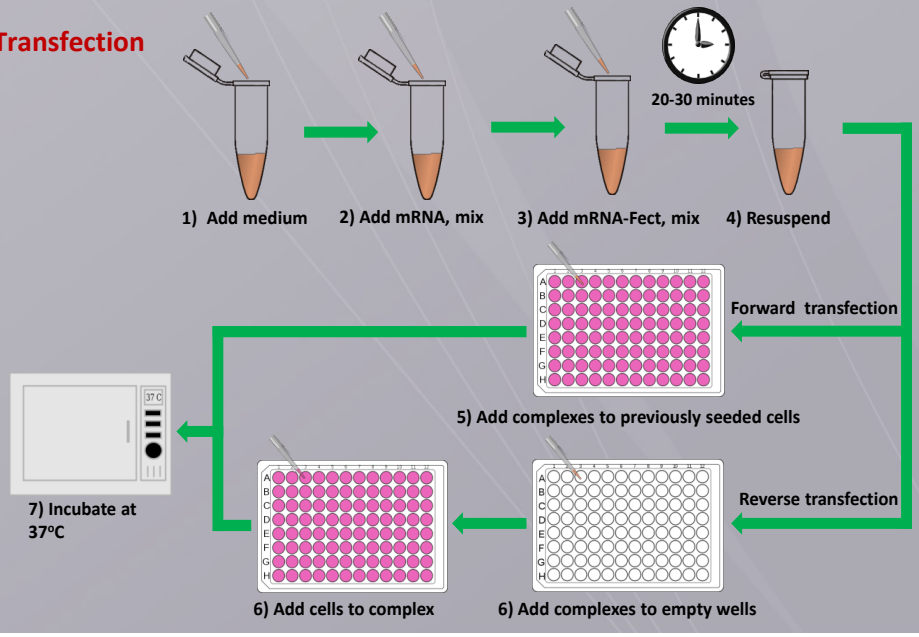
* Recommended volumes for transfection in a single well, assuming 0.1 mg/mL mRNA and 1 mg/mL **mRNA-Fect ULTRA** solutions (mRNA: **mRNA-Fect ULTRA** ratio is 1:8). Complex volumes should be adjusted based on no of replicates.

- 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 ULTRA** 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 previously seeded cells (allowed to attach for 24 hours). 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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