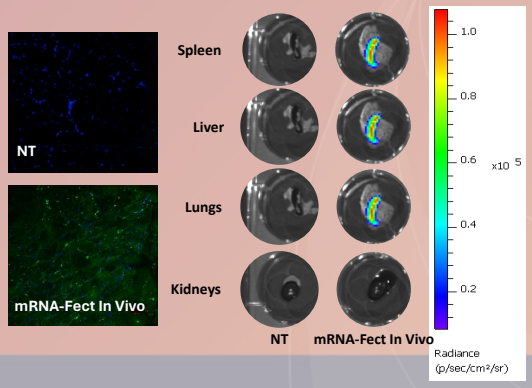
Tailored Transfection Reagent: *mRNA-Fect In Vivo*

PRODUCT NUMBERS: 80-30	SIZE: 1.0 mL	CONCENTRATION: 5.0 mg/mL	STORAGE: -20 °C

Product Description

The **mRNA-Fect In Vivo** reagent is a highly effective transfection reagent optimized for the delivery of a range of mRNA in preclinical animal models. **mRNA-Fect In Vivo** is a synthetic cationic lipopolymer that is tailored primarily for mRNA delivery after extensive testing of cationic lipopolymer 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 In Vivo** occurs in aqueous buffers, obviating the need for organic solvents during preparation. The **mRNA-Fect In Vivo** is a non-integrating carrier, so that the genetic make-up of host cells is not altered after the treatment. The **mRNA-Fect In Vivo** 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 In Vivo** with polynucleotides may need to be optimized for specific cell types and transfection conditions.

GFP expression in tibialis anterior muscles and **Luciferase expression** in different vital organs of NCG mice. NT (No-treatment): Injection with RPMI. mRNA-Fect In Vivo/mGFP or Luc-mRNA: mGFP injection with mRNA-Fect In Vivo formulation as part of the mRNA-Fect In Vivo. The animals were analyzed after 2 days of injection for the gene expression. A robust GFP or Luc expression was evident in the animals treated with reporter gene complexed using the mRNA-Fect.



| 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 recommendations are suggested for preparation of mRNA complexes with the **mRNA-Fect In Vivo** and subsequent delivery to the animal models. Please ensure all reagents are at room temperature for the formulation and injection procedures

- Recommended amounts of **mRNA** and **mRNA-Fect In Vivo** are shown in the Table below for different amounts of mRNA administration. The final recommendations for mRNA amount are 5 to 20 µg per injection and 37.5 to 150 µg per injection for the **mRNA-Fect In Vivo**.
- The recommended ratio of nucleic acid: mRNA-Fect In Vivo is 1.0:7.5, but this ratio can be adjusted depending on the cell type to be modified or administration route.
- A 5 µg of mRNA injection is usually sufficient to obtain local effects while a larger amount such as 20 µg might be needed for systemic injections. The exact amount will depend on the efficacy of the mRNA coded protein.

mRNA (µg)	Buffer (µL)	mRNA (µL) *	mRNA-Fect In Vivo (µL)	Volume/Injection (µL)
5.0	37.5	5.0	7.5	50.0
10.0	25.0	10.0	15.0	50.0
20.0	50.0	20.0	30.0	100.0

* Recommended volumes to make transfection complexes if using 1 mg/mL mRNA and 5 mg/mL mRNA-Fect In Vivo. This ratio will give mRNA: mRNA-Fect In Vivo ratio of 1:7.5.

- 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

- Add the appropriate volume of a buffer into a sterile 1.5 mL Eppendorf tube.
- Add a desired volume of mRNA solution to the polymer solution to the 1.5 mL Eppendorf tube above and vortex gently for 3 sec.
- Add mRNA-Fect In Vivo solution to the mRNA solution. Vortex for 3 sec and incubate for 20-30 min.
- Re-suspend the complexes in solution using a pipette at the end of the incubation period.
- Withdraw complexes into a suitable size syringe (e.g., 28G) and inject subcutaneously (SC), intravenously (IV) or intraperitoneally (IP) depending on the desired administration route.
- Follow the approved animal ethics protocol for subsequent animal handling and maintenance.

| Graphical Procedure for preparation of pDNA transfection complexes and administration