mRNA-In™

Transfection Reagent "Quick Start Protocol"

Overview

mRNA-In™ Reagent is a formulation of chemically defined compounds, and is completely free of animal-derived components. **The protocol provided below has been optimized to achieve the highest number of cells transfected in a population (%CT), without toxicity**. **Unmodified mRNA can be extremely toxic at high concentration and lower amounts of unmodified mRNA should be used depending on the cell type.** It's recommended that the first set of experiments be done using a GFP mRNA to optimize percent cells transfected with mRNA-In™ Reagent. The amount of mRNA/mRNA-In™ Reagent complex that is added to cells is a critical factor in determining percent cells transfected, level of expression, and cellular toxicity. mRNA-In™ Reagent has been optimized for intracellular delivery of mRNA into cultured mammalian cells in the presence of serum at a cell density of 60% to 70%. The reagent can be used with less or more mRNA depending on the level of expression required. High %CT can be achieved with low levels of mRNA.

Storage & Stability

- mRNA-In™ Transfection Reagent is shipped at room temperature. Store at 4°C. DO NOT FREEZE!

Materials Required

- mRNA-In ™ Reagent
- Opti-MEM® I[1] Reduced Serum Medium , other serum free medium, or PBS-Mg++/Ca++
- Cells -70% confluence in media with no heparin
- mRNA (*not supplied*) modified or unmodified.

IMPORTANT NOTES – **Before You Start**

- **Antibiotics** - Do not add antibiotics to medium during transfection as this leads to cell death.
- **Transfection Optimization** - The optimal concentrations of mRNA-In™ Transfection Reagent and mRNA should be determined empirically for each cell line (*see section Optimization and Scaling*).
- **mRNA Concentrations** - Cytotoxicity is greatly influenced by the amount of mRNA present and the optimal amount should be determined. The lowest concentration which provides adequate expression should be used. **If toxicity is observed, reduce the amount of mRNA used**. Lowering the amount mRNA used does not reduce the % cells transfected in general.

Transfection Protocol

This protocol is written for transfection of cells in a 24-well plating format. It may be adapted to other formats by scaling the volumes up or down to fit the format used (see table next page).

A. Day Before Transfection - Cell Plating Preparation

Approximately 24 hours before transfection, cells should be plated such that the cell density is approximately 70-80% confluent at the time of transfection in complete medium without antibiotics. For a 24-well plate format, cells should be plated in 500µl of medium per well.

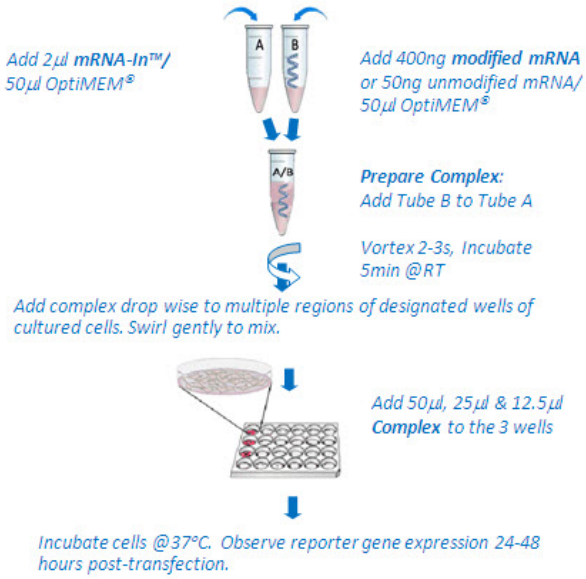
B. Day of Transfection – Transfection Reagent Preparation:

1. Thaw mRNA at room temperature.
2. Allow the **mRNA-In™ Transfection Reagent** to reach room temperature.
3. Mix the reagent by inversion of the tube several times.

C. Day of Transfection – General mRNA Transfection Protocol

1. To 2 sterile tubes marked **A** & **B** add 50µl of Opti-MEM®I medium pre-warmed to room temperature.
2. Add 2µl of mRNA-In™ Reagent to tube **A**.
3. Add **400ng of modified mRNA** to tube **B** or **50 ng of unmodified mRNA** as a starting point to tube B
4. **Complex**: Add diluted mRNA (tube **B**) to tube **A.**

mRNA-In™ Protocol Schematic



5. Mix complex by briefly vortexing. Incubate the complexing mixture at room temperature for 5 minutes.

6. To each of 3 cell-containing wells, add 12.5µl, 25µl, or 50µl* of the complexing reaction to the 500µl of existing medium.
   Note: Please test all the above volumes of complex. *This titration is used to determine the optimal amount of mRNA required.*

7. After an appropriate length of incubation, typically 24-48 hours, measure the transfection efficiency via an assay tailored to the reporter gene that was used.

50 µl of complex is equivalent to 1µl mRNA-In™ Transfection Reagent and 200ng **modified mRNA or 25ng **unmodified** mRNA*

Optimization and Scale-Up

Results from this "Quick Start Protocol" may help guide the optimization process. A good starting point may be the low, middle, or high portions of the preceding recommended ranges and amounts depending on whether best results were observed with the 12.5µl, 25µl, or 50µl transfection complex, respectively, *i.e.,* if the best result was obtained with the 25µl addition (125ng mRNA) this would indicate that optimization experiments should center on 0.125 µg mRNA per 50µl of complex with this particular cell type. Titrate various amounts of the mRNA-In™ Reagent with 0.125ug of mRNA. For a more complete discussion of this topic, please visit our web site at the address below. Different mRNA molecules may require more or less mRNA depending on function.

Table 1 - Recommended quantities for transfecting mRNA in various plate formats.
***IMPORTANT NOTE:** Volumes for unmodified mRNA are shown in red/parentheses

Culture Plate	Relative Surface Area (cm^2/well)	Volume of Complete Medium	Volume of mRNA / mRNA-In™ Complex	Recommended *Start Volume* of mRNA	Amount of mRNA for *Optimization*	Volume of mRNA-In™ Reagent
96-well	0.2x	100µl	10µl	0.04µg (5ng)	0.01-0.1µg (1-10ng)	0.05-0.4µl
48-well	0.4x	200µl	20µl	0.1µg (12.5ng)	0.02-0.2µg (2.5-25ng)	0.1-0.8µl
24-well	1x	500µl	50µl	0.2µg (25ng)	0.05-0.5µg (5-50ng)	0.25-4µl
6-well	5x	2.5ml	250µl	1.0µg (125ng)	1-5µg (50-250ng)	0.5-8µl

The amount of mRNA used in forming transfection complexes determines toxicity Unmodified mRNA can be extremely toxic. Optimization involves determining the optimal amount of mRNA along with the best reagent to mRNA ratio. Generally, as a starting point we recommend examining at least **four (4) different mRNA amounts** over an 8- to 10-fold range **matrixed with mRNA-In™ Transfection Reagent over a 4-fold range**. For example, in a **24-well format**, we suggest setting up complexing reactions with **0.125, 0.25, 0.5 and 1.0 µg mRNA per 50 µl** of serum-free medium. For each mRNA amount, **add 0.05, 0.075, 1, 2, or 3µl of mRNA-In™ Transfection Reagent**. As controls, include 'Reagent alone' and 'mRNA alone' added to cell-containing wells. Unmodified mRNA titration range should be 10ng to 100ng.

Do not make mRNA/mRNA-In™ complexes in volumes smaller than 20µl nor handle individual components in volumes of less than 1µl. mRNA-In™ Reagent may be diluted in Opti-MEM®I immediately before use, if needed. Diluted reagent is not stable to storage and should be discarded.

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