Pub. No. MAN0010803 Rev D.0

## Package Contents

| Catalog Number | Size |
|---|---|
| LMRNA001 | 0.1 mL vial |
| LMRNA003 | 0.3 mL vial |
| LMRNA008 | 0.75 mL vial |
| LMRNA015 | 1.5 mL vial |
| LMRNA150 | 15 mL vial |

## Storage Conditions

Store at 4°C (do not freeze).

## Required Materials

- mRNA (0.5–5 µg/µL stock)
- mRNA positive control
- Opti-MEM™ Reduced Serum Medium
- Eppendorf tubes

## Timing

Preparation: 10 minutes
Incubation: 10 minutes, 5 minutes
Final Incubation: 1–3 days

## Selection Guide

Lipofectamine™ Reagents

Go online to view related products.

## Product Description

- Lipofectamine™ MessengerMAX™ mRNA transfection reagent is a proprietary formulation that is optimized to deliver the highest amount of mRNA possible into neurons and a range of difficult-to-transfect primary cells.

## Important Guidelines

- mRNA-Lipofectamine™ MessengerMAX™ complexes must be made in serum-free medium, but can be added directly to cells in culture medium with/without serum/antibiotic.
- It is not necessary to remove complexes or change/add medium after transfection.
- The amount of Lipofectamine™ MessengerMAX™ reagent required for successful transfection varies depending on the cell type and passage number. Start any new transfection by testing the two recommended concentrations of Lipofectamine™ MessengerMAX™ Reagent to determine an optimum amount.

## Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.
25 May 2015

## Protocol Outline

A. Plate cells so they will be 70–90% confluent at the time of transfection.
B. Prepare plasmid mRNA-lipid complexes.
C. Add mRNA-lipid complexes to cells.

## Transfection Amounts

| Component | 96-well | 24-well | 6-well |
|---|---|---|---|
| Final mRNA per well* | 100 ng | 500 ng | 2500 ng |
| Final Lipofectamine™ MessengerMAX™ Reagent per well | 0.15 and 0.3 µL | 0.75 and 1.5 µL | 3.75 and 7.5 µL |

* To help ensure validity of transfection results, we strongly recommend the use of a positive control mRNA available from various third party vendors including TriLink BioTechnologies (e.g. EGFP mRNA).

## mRNA Synthesis

The Ambion™ mMESSAGE mMACHINE™ T7 Ultra Kit (Cat. no. AM1345) is recommended for synthesis of mRNA transcripts incorporating a 5′ ARCA cap and 3′ poly(a) tail.

Any plasmid with the gene of interest regulated by a T7 polymerase promoter can be used as the reaction template.

A PCR product of the gene of interest may also be used. See below for details:

**Forward Primer**

GC (1) TAATACGACTCACTATAGGG (2) ACAG (3) GCCACC (4) ATG (5) (gene specific sequence) (6)

**Reverse Primer**

Use the reverse complement sequence of the gene of interest.

## Primer Characteristics

## Genome Editing Applications

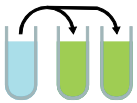
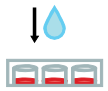
Lipofectamine™ MessengerMAX™ reagent increases the likelihood of successful cleavage and recombination with GeneArt™ CRISPR Nuclease mRNA (Cat. no. A25640) through highly efficient transfection, and ultimately maximizes the efficiency of genetic modifications and simplifies downstream processes.

## Scaling Up or Down Transfections

## Limited Product Warranty and Disclaimer Details

Transfect cells according to the following table. Use the indicated volume of transfection reagent at the two recommended doses as a starting point for optimization. Volumes in each column are for a single well. Scale the volumes proportionally for additional wells.

Each reaction mix volume is for one well and accounts for pipetting variations.

| Timeline | | Steps |
|---|---|---|
| Day 0 | 1  | **Seed cells to be 70–90% confluent at transfection** |
| Day 1 | 2   | **Dilute MessengerMAX™ Reagent in Opti-MEM™ Medium (2 tubes) – Mix well** |
| | 3   | **Incubate** |
| | 4   | **Prepare Diluted mRNA master mix by adding mRNA to Opti-MEM™ Medium – Mix well** |
| | 5  | **Add Diluted mRNA to each tube of Diluted MessengerMAX™ Reagent (1:1 ratio)** |
| | 6   | **Incubate** |
| | 7  | **Add mRNA-lipid complex to cells** |
| Day 2–3 | 8  | **Visualize/analyze transfected cells** |

| Procedure Details (Two Reaction Optimization) | | | |
|---|---|---|---|
| Component | 96-well | 24-well | 6-well |
| Adherent cells | 1–4 × $10^4$ | 0.5–2 × $10^5$ | 0.25–1 × $10^6$ |
| Opti-MEM™ Medium | 5 µL × 2 | 25 µL × 2 | 125 µL × 2 |
| Lipofectamine™ MessengerMAX™ Reagent | 0.15 and 0.3 µL | 0.75 and 1.5 µL | 3.75 and 7.5 µL |
| Incubate diluted MessengerMAX™ Reagent in Opti-MEM™ Medium for 10 minutes at room temperature. | | | |
| Opti-MEM™ Medium | 10 µL | 50 µL | 250 µL |
| mRNA (0.5–5 µg/µL) | 0.2 µg | 1 µg | 5 µg |
| Diluted mRNA | 5 µL | 25 µL | 125 µL |
| Diluted Lipofectamine™ MessengerMAX™ Reagent | 5 µL | 25 µL | 125 µL |
| Incubate for 5 minutes at room temperature. | | | |
| Component (per well) | 96-well | 24-well | 6-well |
| mRNA-lipid complex | 10 µL | 50 µL | 250 µL |
| mRNA | 100 ng | 500 ng | 2500 ng |
| Lipofectamine™ MessengerMAX™ Reagent | 0.15 and 0.3 µL | 0.75 and 1.5 µL | 3.75 and 7.5 µL |
| Incubate cells for 1–2 days at 37°C. Then, analyze transfected cells. | | | |