Lipofectamine™ MessengerMAX™ Reagent

Lipotectaillille MessellyerMAX Reagen

by Thermo Fisher Scientific

ı		
ı	⋘	
ı		

Package Contents

Pub. No. MAN0010803 Rev D.0

Catalog Number Size

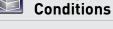
LMRNA001 0.1 mL vial

LMRNA003 0.3 mL vial
 LMRNA008 0.75 mL vial
 LMRNA015 1.5 mL vial

• LMRNA150 15 mL vial



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.

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 TAATACGACTCACTATAGGG ACAG GCCACC ATG (gene specific sequence)

Reverse Primer

Use the reverse complement sequence of the gene of interest.

(1) 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.

- **1** Scaling Up or Down Transfections
- 1 Limited Product Warranty and Disclaimer Details

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

Lipofectamine™ MessengerMAX™ Reagent mRNA Transfection Protocol

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		Timeline	Steps	
Day 0	1		Seed cells to be 70–90% confluent at transfection	
Day 1	2	Diluted MessengerMAX™ Reagent Vortex 2–3 sec	Dilute MessengerMAX™ Reagent in Opti-MEM™ Medium (2 tubes) – Mix well	
	3	10	Incubate	
	4	Diluted mRNA	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	5	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	$1-4 \times 10^4$	$0.5-2 \times 10^5$	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		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 μ g/ μ L)	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.								