









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		Steps
Day 0	1 	Seed cells to be 70–90% confluent at transfection
	2  Diluted MessengerMAX™ Reagent Vortex 2–3 sec	Dilute MessengerMAX™ Reagent in Opti-MEM™ Medium (2 tubes) – Mix well
Day 1	3 	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 	Incubate
	7 	Add mRNA-lipid complex to cells
	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.			