

**Transfectamine™ mRNA Transfection Reagent**

 Catalog number: 60030, 60031  
 Unit size: 0.5 mL, 5 mL

Component	Storage	Amount (Cat No. 60030)	Amount (Cat No. 60031)
Transfectamine™ mRNA Transfection Reagent	Freeze (< -15 °C), Minimize light exposure	1 vial (0.5 mL)	1 vial (5 mL)

**OVERVIEW**

Transfectamine™ mRNA Transfection Reagent is a powerful and versatile transfection reagent designed to introduce a higher amount of mRNA into eukaryotic cells, or more specifically, into animal cells. It delivers high transfection efficiency in a wide variety of adherent and suspension cell lines, including difficult-to-transfect cells. Nuclear uptake is not required, which results in faster protein expression than DNA transfection without the risk of genomic integration. The low toxicity of Transfectamine™ mRNA Transfection Reagent allows higher viability of transfected cells. Transfectamine™ mRNA Transfection Reagent does not require special medium and is easier to use compared to most of the commercial transfection reagents.

**AT A GLANCE**
**Protocol Summary**

1. Prepare cells for transfection.
2. Prepare the Transfectamine™ mRNA Transfection Reagent-RNA mixture.
3. Add the Transfectamine™ mRNA Transfection Reagent-RNA mixture to the cell culture.
4. Culture cells overnight.
5. Analyze transfection efficiency with an appropriate method.

**CELL PREPARATION**

1. Culture cells to ~ 90% confluency at the time of transfection.
2. Replace with fresh growth medium before transfection. For example, replace with 2 mL of medium per well for 6-well plates and 6 mL of medium for 10 cm plates.

**PREPARATION OF WORKING SOLUTION**
**Transfectamine™ mRNA Transfection Reagent-RNA mixture**

1. Mix 2.5 µg of mRNA with 200 µL of serum-free medium.
2. Add 7.5 µL of Transfectamine™ mRNA Transfection Reagent to Step 1.
3. Mix well and incubate at RT for 20 minutes.

**Note:** The ratio of Transfectamine™ mRNA Transfection Reagent to mRNA needs to be optimized for different cell lines. In general, Transfectamine™ mRNA Transfection Reagent (µL) to mRNA (µg) Ratio = (3 to 5 µL) to 1 µg.

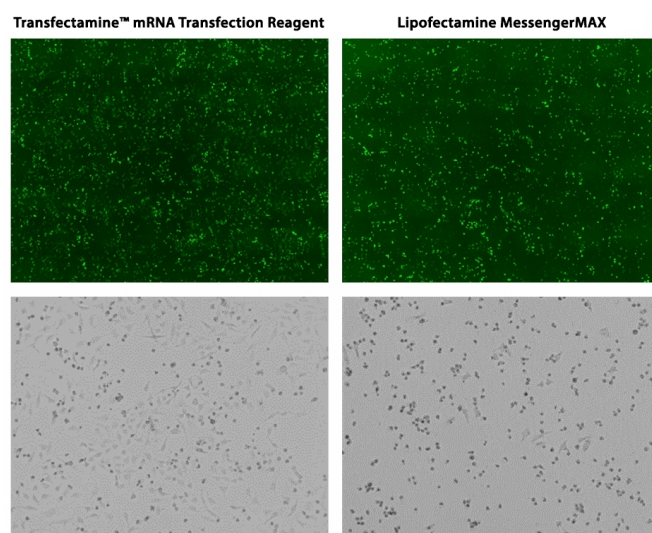
**Table 1.** Sample protocol detail for 6-well plates as shown in the table below.

Component	6-well plate (per well)
Fresh culture medium	2 mL
Purified mRNA	~2.5 µg
Serum-free medium	200 µL
Transfectamine™ mRNA Transfection Reagent	~7.5 µL

**SAMPLE EXPERIMENTAL PROTOCOL**
**Transfection Protocol**

1. Add the Transfectamine™ mRNA Transfection Reagent -mRNA mixture to the culture plate and culture overnight.

**Note:** Recombinant protein expression can be detected as early as 8 hours after the transfection. Maximal expression level may be observed ~24 hours after the transfection.

**EXAMPLE DATA ANALYSIS AND FIGURES**


**Figure 1.** Transfection efficiency comparison (Upper panel) and cellular toxicity comparison (Bottom panel) in HeLa cells. HeLa cells were cultured in a 6-well plate to ~90% confluency. 2.5 µg of mRNA was transfected with Lipofectamine MessengerMAX and Transfectamine™ mRNA Transfection Reagent, respectively. Images were taken 18 hours after the transfection using a fluorescent microscope with the FITC channel (Upper panel). Although transfection efficiency was similar for Lipofectamine MessengerMAX and Transfectamine™ mRNA Transfection Reagent, most Lipofectamine MessengerMAX transfected samples were scrambled,

whereas cells transfected with Transfectamine™ mRNA Transfection Reagent looked much healthier (bottom panel).

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