
Catalog number C15053-K01/ C15053-K02

Package & Component	Reagents	Form	C15053-K01	C15053-K02
	<i>EndoSafe mRNA Reagent</i>	Liquid	0.5 mL*1	0.5 mL*2
	<i>mRNA TurboMix</i>	Liquid	0.5 mL*1	0.5 mL*2

Product Description

The purpose of the EndoSafe mRNA Transfection Kit is to transfect RNA into a variety of cell types with the least amount of cellular damage possible. By delivering the RNA straight to the cytoplasm for expression, RNA delivery overcomes the effects of transcriptional control. mRNAs can be delivered using the EndoSafe mRNA Transfection Kit. It has several uses, including investigations on short-term protein expression, viral production and replication studies.

EndoSafe mRNA Reagent and mRNA TurboMix are the two components of the mRNA transfection kit. The transfection efficiency is highest when serum is present, and this kit is compatible with serum. It also has minimal toxicity to the target cells, preventing cell death during transfection.

Storage & Stability

This product is stable after storage at:

- 20°C or -80°C for 12 months under sterile conditions from date of receipt.
- 2-8°C for 6 months under sterile conditions from date of receipt.

Avoid repeated freeze/thaw cycles.

Materials Required but not Provided

Devices & Consumables

- 10 mL graduated pipettes
- 10 µL to 1000 µL adjustable single-channel micropipettes with disposable tips
- Disposable microcentrifuge tubes
- Timer
- Incubator capable of maintaining temperature at 37±1°C
- Disposable gloves
- Discard container for bio-medical waste

Reagents

- Cultured cells
- Appropriate cell culture medium
- Purified RNA
- Serum-free medium
- Reporter assay as required

Intended Use

The EndoSafe mRNA transfection kit is typically intended for introducing exogenous mRNA molecules into cells for various purposes, such as gene expression studies, protein production, or gene therapy research. The kit's reagents efficiently transport mRNA into target cells with minimal toxicity, ensuring high transfection efficiency. Researchers often use mRNA transfection kits in molecular biology and biotechnology experiments to manipulate gene expression levels in cells temporarily without altering the genome permanently.

The following procedure outlines how to conduct RNA transfections in 24-well plates. As the surface areas of different containers vary, adjustments in the quantities of serum-free medium, EndoSafe mRNA Reagent, mRNA TurboMix, RNA, and complete culture medium are necessary based on the container's surface area.

Transient RNA Transfection Protocol for Each Well of a 24-Well Plate**A. Plate cells**

1. Approximately 18–24 hours before transfection, plate cells in 1.0 ml complete growth medium per well in a 24-well plate. Cells should ideally be $\geq 80\%$ confluent prior to transfection.

For adherent cells

Plate cells at a density of $0.8\text{--}3.0 \times 10^5$ cells/ml.

2. Incubate the cell cultures overnight

B. Prepare Reagents: EndoSafe mRNA Reagent + mRNA TurboMix + RNA (Immediately Before Transfection)

1. Remove the existing culture medium from the 24-well plate and replace it with 0.5 mL serum-free medium

**If the experiment requires, you can opt for the use of complete growth medium by employing fresh 10% FBS medium. However, these variations also need to align with the experimental and target cell requirements.*

2. (Tube A) Mix a 1.5 μL of EndoSafe mRNA Reagent and 1.5 μL of mRNA TurboMix with 25 μL of serum-free medium in a sterile Eppendorf tube.
3. (Tube B) Prepare 1 μg of RNA (1 μL of a 1 $\mu\text{g}/\mu\text{L}$ stock) in 25 μL of serum-free medium. Mix gently by pipetting to ensure complete dissolution.
4. Combine the Tube A and B. Gently pipet to thoroughly mix the components, then allow the mixture to incubate for 10 minutes.

** Please refer to Table 1 & 2 at the end for adjusting reagent volumes based on the plate used in your experiment.*

C. Distribute the Complexes to Cells and Monitor Results

1. Add the solution from Step B to the cells for transfection. Three hours later, switch out the serum-free medium for complete

Procedures

medium that contains serum.

2. To equally distribute the reagents and mRNA, gently shake the culture dish back and forth and side to side.
3. Observe the results at 24 hours and 48 hours post-transfection.

**Note that observations of results at 24- and 48-hours post-transfection may vary depending on the nature of the transfected RNA and the objectives of the experiment.*

Table1. Recommended initial serum-free medium volume for cell culture in Step 1 of Procedure B.

Culture dish	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Surface area	0.35 cm ²	1 cm ²	1.9 cm ²	3.8 cm ²	9.6 cm ²
Serum-free medium	0.1 mL	0.3 mL	0.5 mL	1 mL	2 mL

Table2. Recommended starting conditions for mRNA Transfection Kit reagent preparation for Tube A and B in Procedure B.

Culture Plate	96-well plate		48-well plate		24-well plate		12-well plate		6-well plate	
	A	B	A	B	A	B	A	B	A	B
Surface area	0.35 cm ²		1 cm ²		1.9 cm ²		3.8 cm ²		9.6 cm ²	
Serum-free medium	4.6 µL	4.6 µL	13.2 µL	13.2 µL	25 µL	25 µL	50 µL	50 µL	126 µL	126 µL
RNA (1µg/ µl stock)	-	0.18 µL	-	0.5 µL	-	1 µL	-	2 µL	-	5 µL
EndoSafe mRNA Reagent	0.3 µL	-	0.8 µL	-	1.5 µL	-	3 µL	-	5 µL	-
mRNA TurboMix	0.3 µL	-	0.8 µL	-	1.5 µL	-	3 µL	-	5 µL	-

*****The volumes of EndoSafe mRNA Reagent and mRNA TurboMix can be adjusted according to experimental needs. Please note the ratio of RNA to EndoSafe mRNA Reagent and mRNA TurboMix should be no less than 2:1:1.**

For Research Use Only.

Precautions & Warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those persons who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.
- This product is intended for Research use only and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.