LipoReady™ mRNA Transfection Kit

Introductions

LipoReady™ mRNA Transfection Kit is a cationic polymer-based transfection kit designed for in vitro cellular delivery of RNA, including mRNA, self-amplifying RNA (saRNA), and circular RNA (circRNA). The system is compatible with conventional cell lines, primary cells, and neuronal cells, and provides good biocompatibility, high transfection efficiency, and low cytotoxicity. The resulting RNA-transfection reagent complexes can be added directly to the culture medium and are compatible with serum-containing conditions. In most cases, there is no need to remove the complexes or replace the medium immediately after transfection; fresh medium may be supplied 4-6 hours later, depending on the condition of the cells. The kit is easy to use and is suitable for multi-well plates and high-throughput cell-based screening. Because optimal conditions may vary with the cell type and RNA, the recommended amounts in this protocol should be used as starting points for small-scale optimization.

Intended Use: For in vitro transfection of RNA, including mRNA, saRNA, and circRNA, into commonly used eukaryotic cells.

Components and Storage

Size / Components	100 T (12 well plate: 100 wells; 50 μg mRNA)	500 T (12 well plate: 500 wells; 250 μg mRNA)	Storage
LipoReady™ mRNA Transfection Reagent	1 mL	1 mL × 5	2-8 °C
LipoReady™ mRNA Transfection Buffer	5 mL	25 mL	2-8 °C

Shipping: wet ice Storage: 2-8°C (Freezing is not recommended) Shelf life: 1 year

Protocol

1. Seed the cells so that they are 70%-80% confluent at the time of transfection.

***Note:** Transfection efficiency is affected by the cell type, culture medium, RNA characteristics, and other factors. For initial use, test a range of conditions to determine the optimal amount.

2. Thaw an appropriate amount of the mRNA stock solution. The mRNA should preferably be prepared in RNase-free water at a concentration of at least 200 ng/μL. Dilute the mRNA to approximately 50 ng/μL with LipoReady™ mRNA Transfection Buffer. Do not substitute PBS or cell culture medium for LipoReady™ mRNA Transfection Buffer.

***Note:** Example: Add 8 μL of LipoReady™ mRNA Transfection Buffer to 2 μL of a 200 ng/μL mRNA stock solution to obtain an mRNA concentration of 50 ng/μL.

3. Equilibrate the reagents to room temperature and mix gently. Combine equal volumes of LipoReady™ mRNA Transfection Reagent and the diluted mRNA. Immediately mix by gently pipetting 2-3 times, and incubate at room temperature for 2-3 minutes. Avoid vigorous vortexing during complex preparation.

***Note:** Example for one well of a 12-well plate: Combine 10 μL of diluted mRNA with 10 μL of LipoReady™ mRNA Transfection Reagent. Mix gently immediately, incubate at room temperature for 2-3 minutes, and then add to the cells.

4. Add the mRNA-transfection reagent complexes dropwise to the cell culture medium and mix gently (recommended starting amount for a 12-well plate: 0.5 μg mRNA per well).

5. Return the culture plate to the incubator. Gene expression can generally be analyzed 24-96 hours after transfection. The optimal time point should be determined according to the expression kinetics of the target mRNA and the condition of the cells.

***Note:** Use the prepared complexes within 30 minutes. If transfection performance is suboptimal, shake the complexes at 900 rpm and 25°C for 30 seconds, incubate for approximately 5 minutes, and then add to the cells.

Recommended Transfection Amounts for Different Culture Formats

Culture Format	Surface Area	Volume Per-Well		mRNA Transfection		
		Medium Volume per Well	mRNA-Transfection Reagent Complex (Total Volume, μL)	mRNA Amount (μg/well or dish)	Diluted mRNA Volume (μL)	LipoReady™ mRNA Transfection Reagent Volume (μL)
96-well plate	0.3 cm²	0.1 mL	4	0.10	2	2
24-well plate	2 cm²	0.5 mL	10	0.25	5	5
12-well plate	4 cm²	1 mL	20	0.50	10	10
6-well plate	10 cm²	2 mL	40	1.0	20	20
60 mm dish	20 cm²	5 mL	100	2.0	50	50
10 cm dish	60 cm²	15 mL	200	5.0	100	100

***Note:** The mRNA volumes in the table are calculated using an mRNA concentration of 50 ng/μL. The total volume of the mRNA-transfection reagent complex is the sum of the diluted mRNA volume and the LipoReady™ mRNA Transfection Reagent volume. The amounts are provided for reference only and may be scaled proportionally according to culture surface area. Optimize the mRNA amount, mRNA-to-transfection reagent ratio, and analysis time according to the cell type, culture medium, and mRNA characteristics.

Note

1. Use RNase-free consumables and reagents.
2. Store at 2-8°C. Gently mix after opening and equilibrate to room temperature before use.
3. Avoid repeated / prolonged opening. Aliquot to prevent contamination; protect from light for long-term storage.
4. For first use, optimize the nucleic acid/reagent ratio for maximal transfection efficiency.
5. RNA stock concentration >200 ng/μL is recommended; dilute with LipoReady™ mRNA Transfection Buffer.
6. Antibiotics do not prevent transfection but may affect efficiency; optimize as needed.

7. No medium change is required before transfection; adjust according to cell nutritional status.

8. For Research Use Only.

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