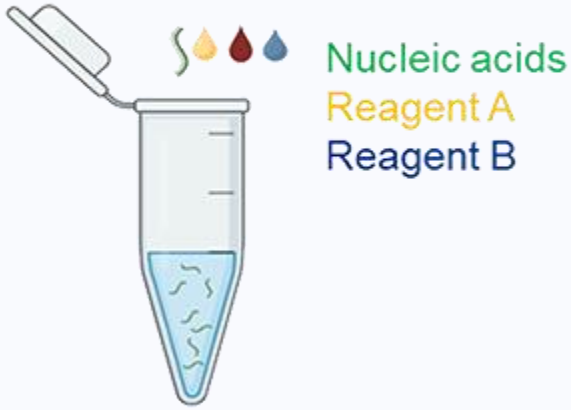
Transfection Protocol for mRNA per Well of a 96-Well Plate

Scheme	Step	Cell Line Transfection Protocol for mRNA per Well of a 96-Well Plate
	1. Transfection Complex Preparation	1.1 Mix Reagent A with mRNA Mix 0.5 µg of mRNA with 40 µL of Reagent A. Note: Invert Reagent A briefly before use to ensure uniformity. 1.2 Add Reagent B Add 1.4 µL of Reagent B to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.

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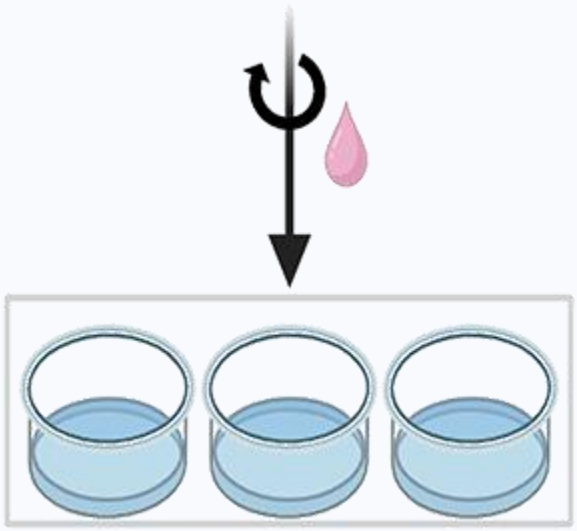
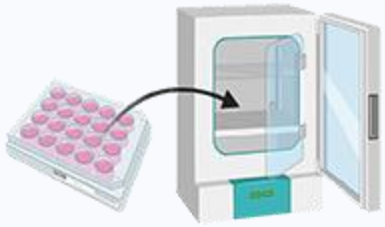
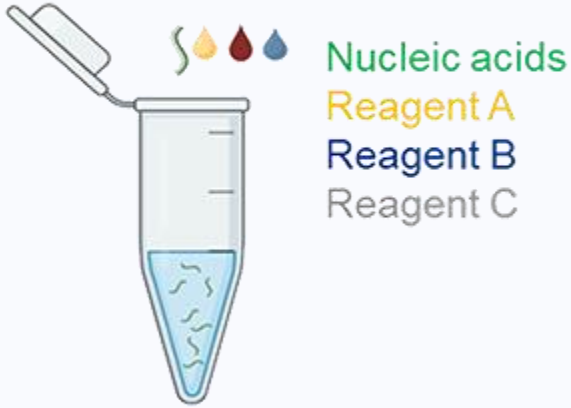
	2. Cell Preparation	2.1 Suspension cells Harvest the cells by centrifugation at 300 g for 5 minutes. Discard the supernatant and wash cells once with Opti-MEM. Resuspend cells with Opti-MEM and adjust concentration to 5×10^6 - 1×10^7 cells/mL. Note: Avoid including FBS in the transfection medium. 2.2 Adherent cells Maintain 50%-80% cell confluence. Remove medium, wash cells once with Opti-MEM, then add 20 µL of Opti-MEM. Note: Avoid including FBS in the transfection medium. Optional: Harvest cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 5×10^6 - 1×10^7 cells/mL for subsequent transfection.
	3. Transfection	3.1 Mix complex with cells For suspension cells, mix 40 µL of transfection complex with 20 µL of cell suspension and gently pipet up and down 2-3 times. For adherent cells, apply directly to the cells. 3.2 Incubation Incubate the cells with the transfection complex for 45-60 minutes in a cell culture

Scheme	Step	Cell Line Transfection Protocol for mRNA per Well of a 96-Well Plate
		incubator. 3.3 Termination Terminate the reaction by adding ≥200 μL of culture medium (10X cell suspension), centrifuge at 300 g for 5 minutes, and discard the supernatant. For adherent cells, replace the transfection mixture with ≥200 μL of culture medium (10X cell suspension). Note: The cell pellet may adhere to the tube walls. Gently remove the supernatant to minimize cell loss. 3.4 Post-transfection culture Incubate transfected cells in culture medium and assess transfection efficiency after 5 to 48 hours, or at an appropriate time.
Scheme	Step	Primary Cell Transfection Protocol for mRNA per Well of a 96-Well Plate

Scheme	Step	Cell Line Transfection Protocol for mRNA per Well of a 96-Well Plate
	1. Transfection Complex Preparation	1.1 Mix Reagent A with mRNA Mix 0.5 µg of mRNA with 40 µL of Reagent A. Note: Invert Reagent A briefly before use to ensure uniformity. 1.2 Add Reagent B Add 0.7 µL of Reagent B to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds. 1.3 Add Reagent C Add 8 µL of Reagent C to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 seconds.

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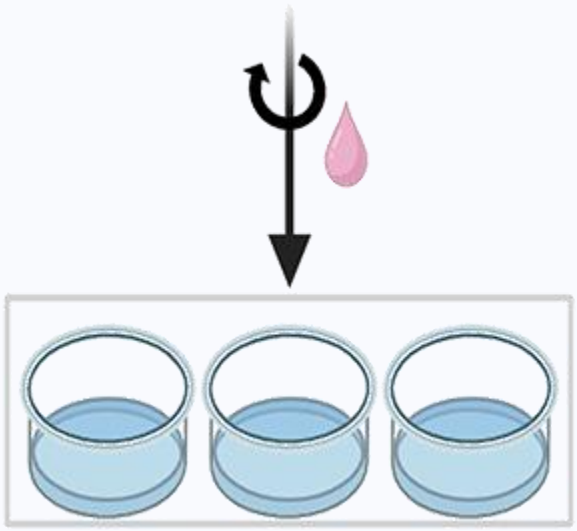
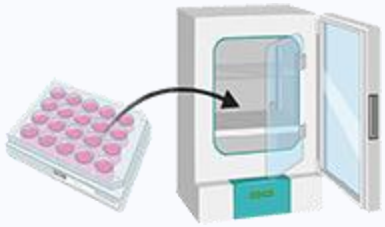
	2. Cell Preparation	2.1 Suspension cells Harvest the cells by centrifugation at 300 g for 5 minutes. Discard the supernatant and wash cells once with Opti-MEM. Resuspend cells with Opti-MEM and adjust concentration to 5×10^6 - 1×10^7 cells/mL. Note: Avoid including FBS in the transfection medium. 2.2 Adherent cells Maintain 50%-80% cell confluence. Remove medium, wash cells once with Opti-MEM, then add 20 µL of Opti-MEM. Note: Avoid including FBS in the transfection medium. Optional: Harvest cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 5×10^6 - 1×10^7 cells/mL for subsequent transfection.
	3. Transfection	3.1 Mix complex with cells For suspension cells, mix 40 µL of transfection complex with 20 µL of cell suspension and gently pipet up and down 2-3 times. For adherent cells, apply directly to the cells. 3.2 Incubation Incubate the cells with the transfection complex for 15-30 minutes in a cell culture

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		incubator. 3.3 Termination Terminate the reaction by adding ≥200 μL of culture medium (10X cell suspension), centrifuge at 300 g for 5 minutes, and discard the supernatant. For adherent cells, replace the transfection mixture with ≥200 μL of culture medium (10X cell suspension). Note: The cell pellet may adhere to the tube walls. Gently remove the supernatant to minimize cell loss. 3.4 Post-transfection culture Incubate transfected cells in culture medium and assess transfection efficiency after 5 to 48 hours, or at an appropriate time.