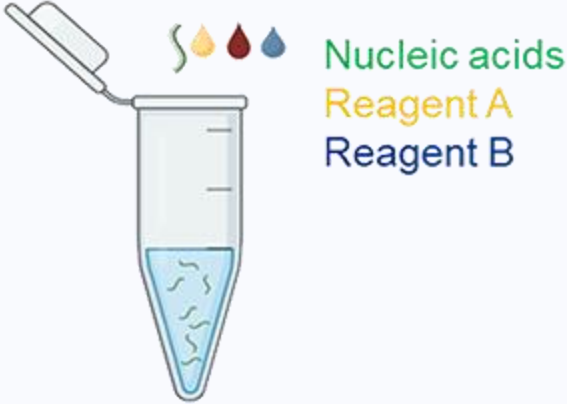
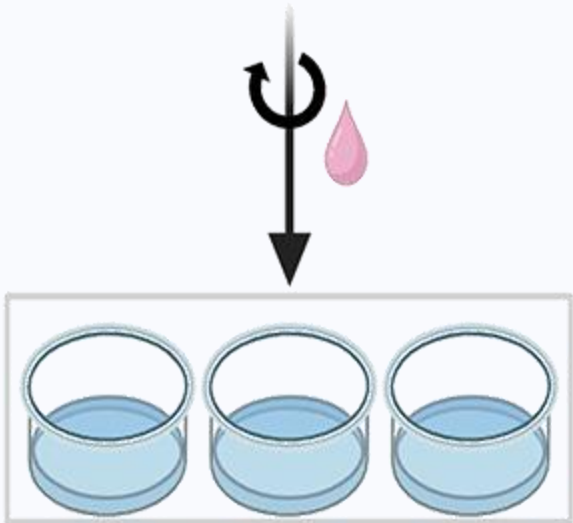
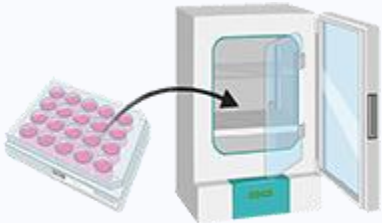


Transfection Protocol for mRNA per Well of a 96-Well Plate

Scheme	Step	
	<p>1. Transfection Complex Preparation</p>	<p>1.1 Mix Reagent A with mRNA Mix 0.5 μg of mRNA with 40 μL of Reagent A. Note: Invert the Reagent A briefly before use to ensure uniformity.</p> <p>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.</p>

Scheme	Step	
	<p>2. Cell Preparation</p>	<p>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.</p> <p>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.</p>
	<p>3. Transfection</p>	<p>3.1 Mix transfection 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.</p> <p>3.2 Incubation Incubate the cells with the transfection complex for 45-60 minutes in a cell culture incubator.</p>

Scheme	Step	
		<p>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.</p> <p>3.4 Post-transfection culture Incubate the transfected cells in culture medium and assess transfection efficiency after 5 to 48 hours, or at an appropriate time.</p>