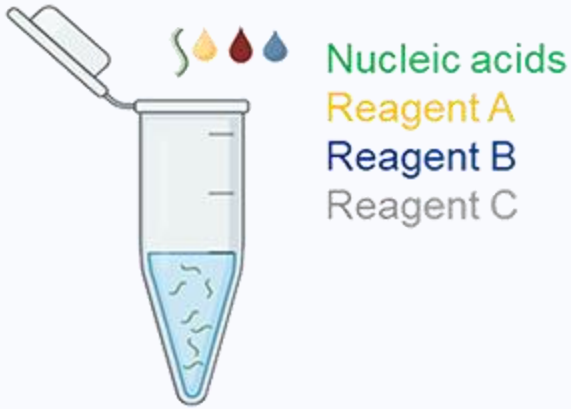
Transfection Protocol for mRNA per Well of a 96-Well Plate

Scheme	Step	
	1. Transfection Complex Preparation b	1.1 Mix Reagent A (for Mouse Immunocyte) with mRNA Mix 0.5 μg of mRNA with 40 μL of Reagent A (for Mouse Immunocyte). Note: Invert Reagent A (for Mouse Immunocyte) briefly before use to ensure uniformity. 1.2 Add Reagent B (for Mouse Immunocyte) Add 0.7 μL of Reagent B (for Mouse Immunocyte) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds. 1.3 Add Reagent C (for Mouse Immunocyte) Add 10 μL of Reagent C (for Mouse Immunocyte) to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 seconds. Note: If precipitation occurs in Reagent C (for Mouse Immunocyte), heat to 65°C until fully dissolved before use.

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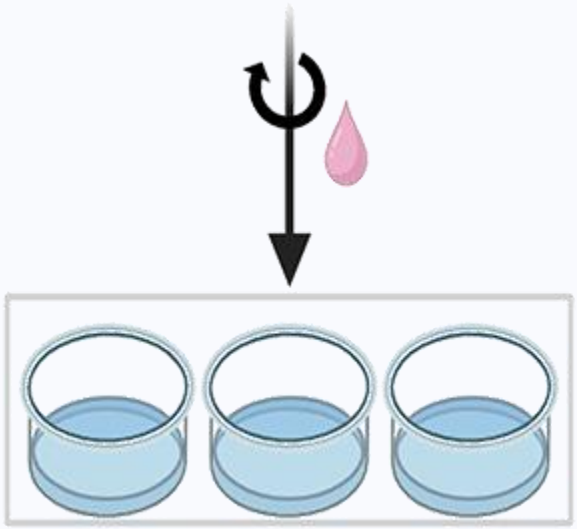
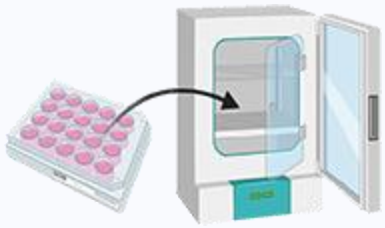
	2. Cell Preparation	2.1 Suspension cells Harvest the cells by centrifugation at 300 g for 5 minutes. Discard the supernatant and wash the cells once with Opti-MEM. Resuspend the cells with Opti-MEM and adjust the cell concentration to 1×107 – 1.5×10^7 cells/mL. Note: Avoid including FBS in the transfection medium. 2.2 Adherent cells Maintain 50%-80% cell confluence. Remove medium, wash the cells once with Opti-MEM, and then add 20 µL of Opti-MEM. Note: Avoid including FBS in the transfection medium. Optional: Harvest the cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 1×107 – 1.5×10^7 cells/mL for subsequent transfection.
	3. Transfection	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 it directly onto the cells. 3.2 Incubation Incubate the cells with the transfection

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		complex for 15-30 minutes in a cell culture 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 the transfected cells in culture medium and assess transfection efficiency after 5 to 48 hours, or at an appropriate time.