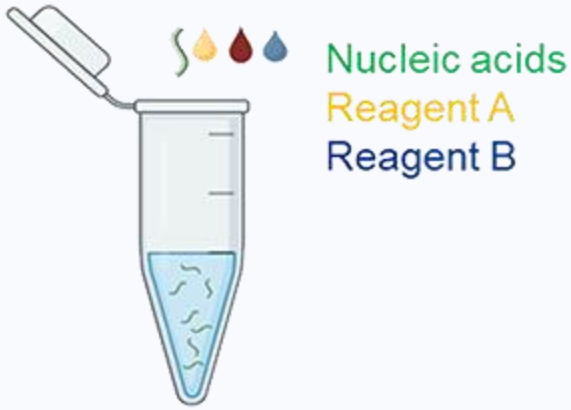
Transfection Protocol for mRNA per Well of a 96-Well Plate

Scheme	Step	Transfection Protocol for ProteanFect™ CRISPR Gene Editing in Various Cell Lines per Well of a 96-Well Plate
	1. Transfection Complex Preparation	1.1 Mix Reagent A (for CRISPR-Cas9 mRNA) with mRNA Mix 0.25 µg Cas9 mRNA and 0.25 µg sgRNA with 40 µL of Reagent A (for CRISPR-Cas9 mRNA). Note: Invert the Reagent A (for CRISPR-Cas9 mRNA) briefly before use to ensure uniformity. 1.2 Add Reagent B (for CRISPR-Cas9 mRNA) Add 1.4 µL of Reagent B (for CRISPR-Cas9 mRNA) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.

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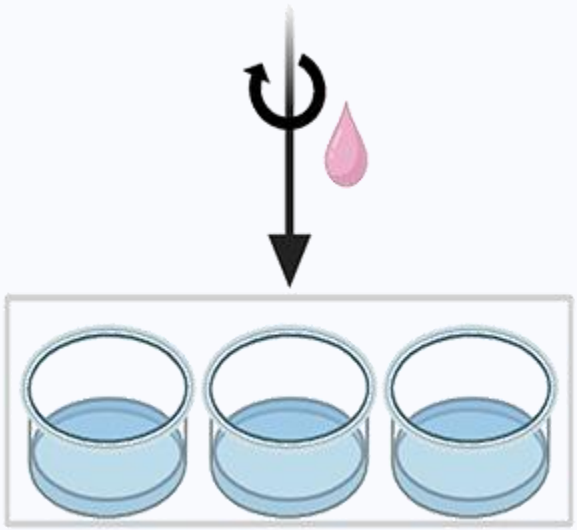
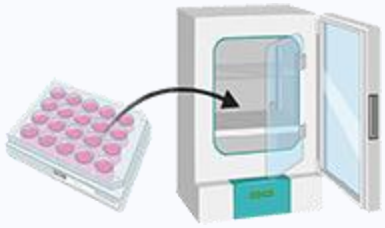
	2. Cell Preparation	2.1 Suspension cells Harvest the cells by centrifugation at 300 g for 5 minutes. Discard the supernatant and wash cells once with Opti-MEM. Resuspend cells with Opti-MEM and adjust concentration to 5×10^6 - 1×10^7 cells/mL. Note: Avoid including FBS in the transfection medium. 2.2 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 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. 3.2 Incubation

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		Incubate the cells with the transfection complex for 45-60 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 evaluate the editing efficiency of the target genes after 48 to 72 hours, or at an appropriate time.