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

| Scheme | Step | Transfection Protocol for ProteanFect™ CRISPRMax Mouse Immunocyte Gene Editing Transfection Kit in Primary mouse immunocyte per Well of a 96-Well Plate |
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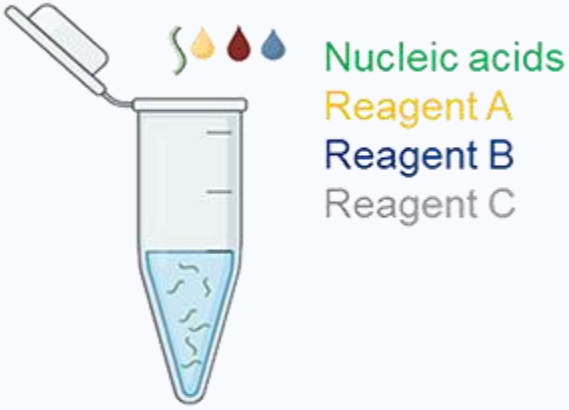
|  | 1. Transfection Complex Preparation | 1.1 Mix Reagent A (for Mouse Immunocyte CRISPR-Cas9 mRNA) with mRNA<br>Mix 0.25 μg Cas9 mRNA and 0.25 μg sgRNA with 40 μL of Reagent A (for Mouse Immunocyte CRISPR-Cas9 mRNA).<br>Note: Invert Reagent A (for Mouse Immunocyte CRISPR-Cas9 mRNA) briefly before use to ensure uniformity.<br><br>1.2 Add Reagent B (for Mouse Immunocyte CRISPR-Cas9 mRNA)<br>Add 0.7 μL of Reagent B (for Mouse Immunocyte CRISPR-Cas9 mRNA) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.<br><br>1.3 Add Reagent C (for Mouse Immunocyte CRISPR-Cas9 mRNA)Add 10 μL of Reagent C (for Mouse Immunocyte CRISPR-Cas9 mRNA) to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 seconds.<br>Note: If precipitation occurs in Reagent C (for Mouse Immunocyte CRISPR-Cas9 |

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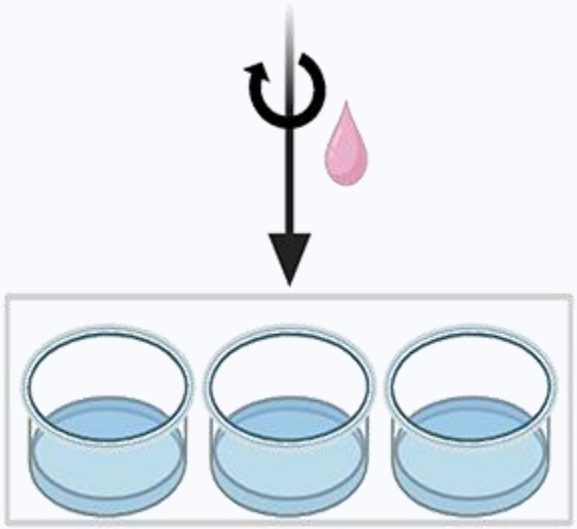
| | | mRNA), heat to 65°C until fully dissolved before use. |
|  | 2. Cell Preparation | 2.1 Suspension cells<br>Harvest the cells by centrifugation at 300 g for 5 minutes. Discard the supernatant and wash the cells once with Opti-MEM. Resuspend the cells with Opti-MEM and adjust the cell concentration to 1×107 – $1.5\times10^7$ cells/mL.<br>Note: Avoid including FBS in the transfection medium.<br><br>2.2 Adherent cells<br>Maintain 50%-80% cell confluence. Remove medium, wash the cells once with Opti-MEM, and then add 20 µL of Opti-MEM.<br>Note: Avoid including FBS in the transfection medium.<br>Optional: Harvest the cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 1×107 – $1.5\times10^7$ cells/mL for subsequent transfection. |

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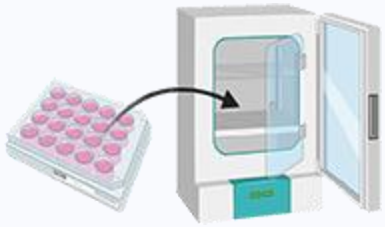
|  | 3. Transfection | 3.1 Mix transfection complex with cells<br>For suspension cells, mix 40 µL of transfection complex with 20 µL of cell suspension and gently pipet up and down 2-3 times.<br>For adherent cells, apply it directly onto the cells.<br><br>3.2 Incubation<br>Incubate the cells with the transfection complex for 15-30 minutes in a cell culture incubator.<br><br>3.3 Termination<br>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).<br>Note: The cell pellet may adhere to the tube walls. Gently remove the supernatant to minimize cell loss.<br><br>3.4 Post-transfection culture<br>Incubate the transfected cells in culture medium and evaluate the editing efficiency |

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| | | of the target genes after 48 to 72 hours, or at an appropriate time. |