sgRNA Usage Instructions

Product Overview

sgRNA is synthesized enzymatically and typically has a length of 100 nt. It is combined with Cas9 protein and introduced into cells via electroporation or chemical transfection reagents for gene editing experiments.

Transportation and Storage

The product is shipped as a lyophilized powder with ice packs. Upon receipt, store the product at -20°C to -80°C. The lyophilized powder can be stably stored for one year. Before use, centrifuge briefly, and prepare a 100μM storage solution using RNase-free H_2O or TE buffer (refer to the table below). Aliquot the solution to avoid repeated freeze-thaw cycles.

Table 1. Preparation of 100μM Storage Solution

Product Amount	RNase-free H_2O
1.5 nmol	15 μl
5 nmol	50 μl
10 nmol	100 μl

Precautions

1. Since the lyophilized powder adheres as a thin film to the tube walls, briefly centrifuge before use to prevent loss. Aliquots of prepared sgRNA solutions should be stored at -20°C, and for long-term storage, keep at -80°C.
2. To avoid degradation due to external factors (enzymes, pH, temperature), strictly follow RNA handling protocols.
3. Transfection efficiency mainly depends on cell characteristics and the transfection method used; choose the appropriate transfection method based on specific experimental cells.
4. Wear disposable gloves during handling.

Cell Experiment Method (CRISPR RNP Liposome Transfection Method)

1. Plate cells in advance. When cell density reaches 45%-55%, proceed with transfection.

The seeding number should be based on growth rate; rapidly growing cells require fewer cells to be seeded before transfection, adjusting according to observed cell density at transfection time.

2. Use the Lipofectamine 3000 Transfection Kit (Thermo Fisher), divided into System A and System B, which are prepared separately and then mixed for transfection.

System A: Opti-MEM™ I Medium, NLS-Cas9 Nuclease, sgRNA, Cas9 Plus™ Reagent. Vortex mix for 3s, centrifuge briefly at room temperature at 4000 rpm for 3s, and let stand at room temperature for 5min.

System B: Opti-MEM™ I Medium, CRISPRMAX™ Reagent. Vortex mix for 3s, centrifuge briefly at room temperature at 4000 rpm for 3s.

After preparing System B, do not incubate. To achieve optimal transfection efficiency, do not let the diluted CRISPRMAX™ reagent stand for more than 3min.

3. Use a pipette to add all of System A to System B, vortex mix for 3s, centrifuge briefly at room temperature at 4000 rpm for 3s, and incubate at room temperature for 10min.

Ensure that the incubation time does not exceed 20min.

4. Remove the cell culture plates (24-well or 6-well) from the incubator when cells have grown to the appropriate density. Gently add the incubated complex from step (3) to the culture medium, shake evenly in a figure-eight motion 5 times, and place in an incubator at 37°C with 5% CO_2.
5. After 48h of culture, collect cells for knockout efficiency analysis. Observe cell status 24h after adding the complex to the medium.

If the medium turns yellow or cell status deteriorates, replace the transfection medium with fresh, pre-warmed complete medium, and continue culturing for another 24h before collecting cells.

Common Causes of Low Editing Efficiency and Solutions

1. **Low sgRNA Efficiency or Prolonged Storage:** Before starting experiments, determine sgRNA editing efficiency using the T7E1 assay. Select high-efficiency sgRNA for subsequent experiments. If none of the designed sgRNAs meet requirements, redesign sgRNA.
2. **Prolonged Storage or Repeated Freeze-Thaw of sgRNA Solution:** Store sgRNA solutions at -20°C to -80°C. For multiple uses, aliquot the solution. For long-term storage, place at -80°C. If sgRNA is over 6 months old, synthesize new sgRNA.
3. **Short Homology Arms of Donor DNA:** The length of homology arms should correlate with the length of the insertion gene. For large insertions, extend the homology arm length.
4. **Transfection Failure and Low Cell Survival:** Choose an appropriate transfection method based on the specific cells. Avoid transfection if cell status is poor. Re-culture cells and transfect when cell vitality and plating density are optimal.
5. **Improper Ratio of Cas9 Protein to sgRNA:** Optimize the ratio of Cas9 protein to sgRNA. For liposome transfection RNP experiments, the recommended concentrations are 0.26μM Cas9 protein and 0.5μM sgRNA.