

## Transfectamine™ 6000 CRISPR Transfection Reagent

Catalog number: 60023, 60024, 60028  
Unit size: 1 mL, 5 mL, 50 uL

Component	Storage	Amount (Cat No. 60023)	Amount (Cat No. 60024)	Amount (Cat No. 60028)
Transfectamine™ 6000 CRISPR Transfection Reagent	Freeze (< -15 °C), Minimize light exposure	1 mL	5 mL	50 uL

### OVERVIEW

Transfectamine™ 6000 CRISPR Transfection Reagent is a non-liposomal formula designed for optimal delivery and effectiveness of CRISPR/Cas9 components across a wide array of cell types. Engineered for targeted genome editing, this reagent demonstrates superior transfection efficiency, particularly in cell lines typically resistant to transfection. The versatility of Transfectamine™ 6000 CRISPR Transfection Reagent supports various CRISPR/Cas9 systems, including plasmids, ribonucleoproteins (RNPs), and mRNA. Its formulation has been optimized to minimize cytotoxicity while maintaining cell viability, ensuring more accurate and reliable experimental results. The straightforward protocol associated with our reagent reduces the need for extensive optimization, streamlining the workflow and conserving valuable time and resources. Transfectamine™ 6000 CRISPR Transfection Reagent is suitable for various applications, such as gene knockout and knock-in experiments, the generation of CRISPR-mediated gene-edited cell lines, functional genomics studies, and research in therapeutic gene editing.

### AT A GLANCE

#### Protocol Summary

1. Prepare cells for transfection.
2. Prepare Transfectamine™ 6000 CRISPR reagent-CRISPR DNA mixture.
3. Add Transfectamine™ 6000 CRISPR reagent-CRISPR DNA mixture to cell culture.
4. Culture overnight.
5. Analyze transfection efficiency with appropriate methods.

### CELL PREPARATION

#### Prepare Cell Culture

1. Culture cells to ~ 80-90% confluency at the time of transfection.
2. Before transfection, replace the old growth medium with a fresh medium. For example, add 2 mL of medium per well for 6-well plates and 6 mL of medium for 10-cm plates.

### PREPARATION OF WORKING SOLUTION

#### Prepare Transfectamine™ 6000-CRISPR DNA Mixture

1. Mix 2.5 µg of DNA with 200 µL of serum-free medium.
2. Add 7.5 µL of Transfectamine™ 6000 CRISPR reagent to Step 1.
3. Mix well and incubate at room temperature for 20 minutes.

**Note:** The recommended Transfectamine™ 6000 CRISPR Transfection Reagent (uL):DNA (ug) ratio is 3-5 µL:1 µg, but

optimization is necessary for different cell lines.

### SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Recommended transfection conditions for 6-well and 10 cm plates using Transfectamine™ 6000 CRISPR Transfection Reagent.

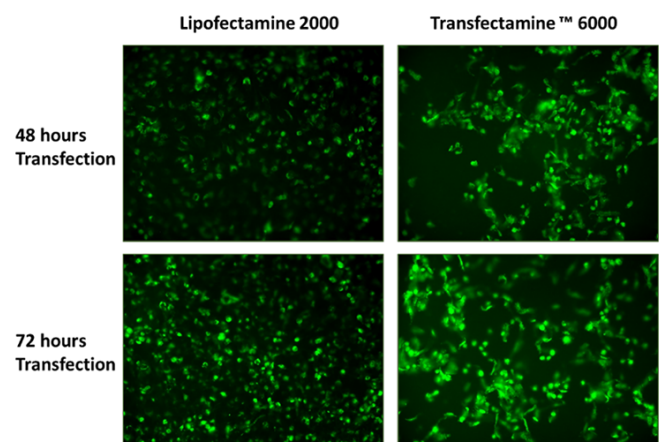
Component	6-well plate (per well)	10 cm plate
Fresh Culture Medium	2 mL	6 mL
CRISPR Plasmid	~2.5 µg	7.5 ~ 10 µg
Serum-free Medium	200 µL	600 µL
Transfectamine™ 6000 CRISPR Transfection Reagent	~7.5 µL	~22.5 µL

#### Transfection Protocol

1. Add Transfectamine™ 6000 CRISPR reagent – CRISPR DNA mixture to culture plate and culture overnight.

**Note:** Recombinant protein expression can be detected as early as 16 hours post-transfection with maximal levels observed between 72-96 hours post-transfection.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Comparison of transfection efficiency in HeLa cells for CRISPR-Cas9-GFP plasmid using Transfectamine™ 6000 CRISPR Transfection Reagent and Lipofectamine 2000. Both reagents were used to transfect HeLa cells in a 96-well format, and GFP expression was analyzed 48 and 72 hours post-transfection. Transfectamine™

6000 CRISPR Transfection reagent provided higher GFP transfection efficiency than Lipofectamine 2000.

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