CRISPR KO Rapid Knockout Kit

User Guide

► Product Information

Catalog No.	Product Name	Specification
EDKO-K04	CRISPR KO Rapid Knockout Kit 2.0 (CRISPR RNP KO Kit 2.0)	50 µL
		100 µL

► Product Overview

The CRISPR KO Rapid Knockout Kit enables efficient and precise gene knockout by delivering pre-assembled ribonucleoprotein (RNP) complexes composed of Cas9 protein and sgRNA, along with a proprietary delivery carrier. Tailored for research use, this ready-to-use kit features EDITGENE's innovative CRISPR RNP delivery system and extensively validated Cas enzymes. The specially treated RNP–carrier complex ensures high-efficiency genome editing in mammalian cells.

► Transportation and Storage

Store at -80 °C. Shipped on dry ice. Shelf life: 6 months.

It is recommended to aliquot the reagents based on usage frequency to avoid repeated freeze-thaw cycles.

► Advantages

- **High Editing Efficiency**

  Utilizes a highly efficient delivery carrier and direct RNP delivery strategy, achieving gene knockout efficiency of up to 95%.

- **Short Turnaround Time**

  The kit provides a ready-to-use carrier–RNP complex. DNA cleavage can be detected as early as 6 hours post-transfection, with complete knockout achieved within 48 hours.

- **Broad Applicability**

  Suitable for most mammalian cell types, including hard-to-transfect cells and cells with limited proliferative capacity.

- **Low Technical Requirements:**

  No complex procedures or electroporation equipment needed. Simply add and use for efficient gene editing. No selection required—thanks to the transient mechanism of action, there's no need for antibiotics or fluorescent markers, significantly shortening the experimental timeline.

- **Low Risk**

  Minimal off-target effects. The RNP complex has a short half-life (a few hours) inside cells, and the Cas protein is rapidly degraded, significantly reducing non-specific cleavage. No risk of random integration of exogenous DNA.

- **Low Cytotoxicity**

  Features an innovative biomolecular delivery carrier combined with RNP delivery to ensure minimal toxicity to cells.

► Components

Catalog No.	Component	Specification	Notes
EDKO-K04-50	Carrier–RNP Complex	50 µL (sufficient for five 24-well reactions)	24-well plate, 10 µL/well
EDKO-K04-100	Carrier–RNP Complex	100 µL (sufficient for five 12-well reactions)	12-well plate, 20 µL/well

Note: This product provides only the pre-assembled carrier–RNP complex. Customers are required to provide their own sgRNA sequences.

► Optional Components

Option	Description
sgRNA Design	Custom sgRNA design services provided by EDITGENE
Positive Control	Carrie-RNP complex targeting human B2M gene

► Experimental Procedure

1. Cell Culture and Seeding (Example: 24-well plate)

Culture cells until they are in a healthy, actively growing state. Seed cells into a 24-well plate 24 hours prior to transfection.

For adherent cells: ensure 50%–60% confluency at the time of transfection.

For suspension cells: ensure a cell density of 1.2×10^5 to 1.6×10^5 cells per well at the time of transfection.

Note: Use healthy cells free from bacterial, fungal, or mycoplasma contamination. For cryopreserved cells recently thawed from liquid nitrogen, passage at least twice before transfection.

2. Cell Transfection

Thaw the carrier–RNP complex slowly by transferring it from –80 °C to 4 °C in advance. Add 10 µL of the complex per well in a 24-well plate. Add the reagent slowly in multiple small aliquots, gently shaking to mix after each addition.

For adherent cells: Cells should be healthy, evenly distributed, and at 50%–60% confluency. The complex can be added directly.

For suspension cells: Cells should be healthy. Gently pipette to disperse cell clumps before adding the carrier–RNP complex. Add the reagent directly once the cells are evenly suspended.

3. Analysis of Transfected Cells

At 48 hours post-transfection, extract genomic DNA from the transfected cells. Use specific primers to amplify the target region (the amplicon should include the sgRNA targeted cleavage site).

Analyze gene editing efficiency using tools such as TIDE (https://tide.nki.nl/) or ICE (https://ice.synthego.com/#/, user guide can be found at: https://www.synthego.com/guide/how-to-use-crispr/ice-analysis-guide).

► High-Efficiency Gene Editing Results

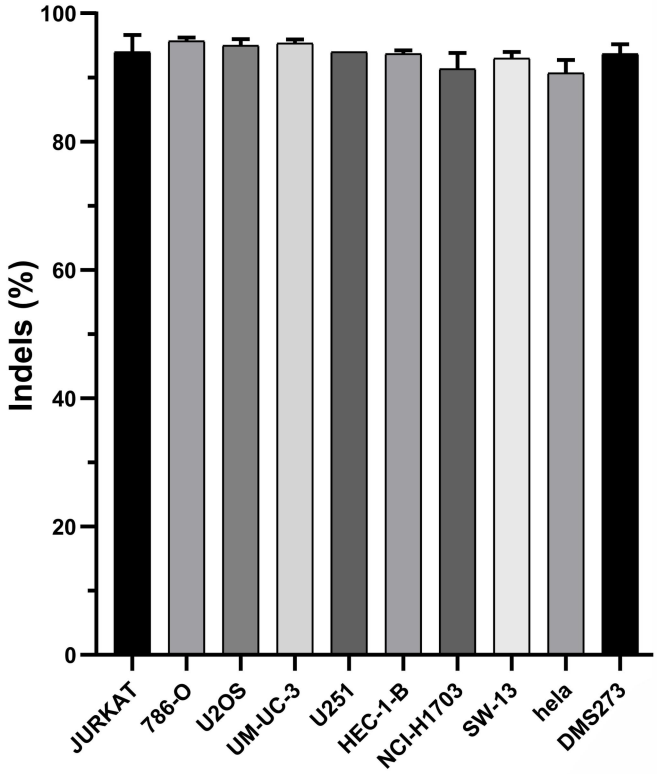

Figure 1. Representative Image of Efficiently Edited Cells

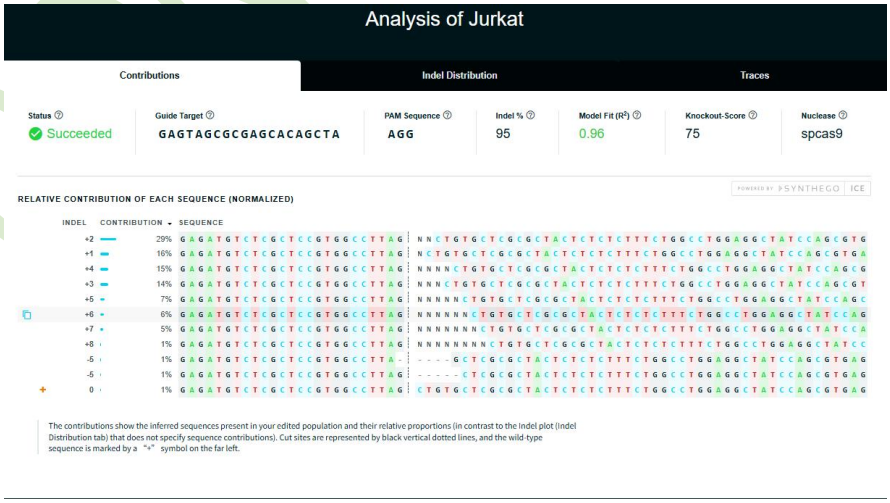

Figure 2. ICE Analysis of Polyclonal Editing in Jurkat Cells (Target: *B2M*)

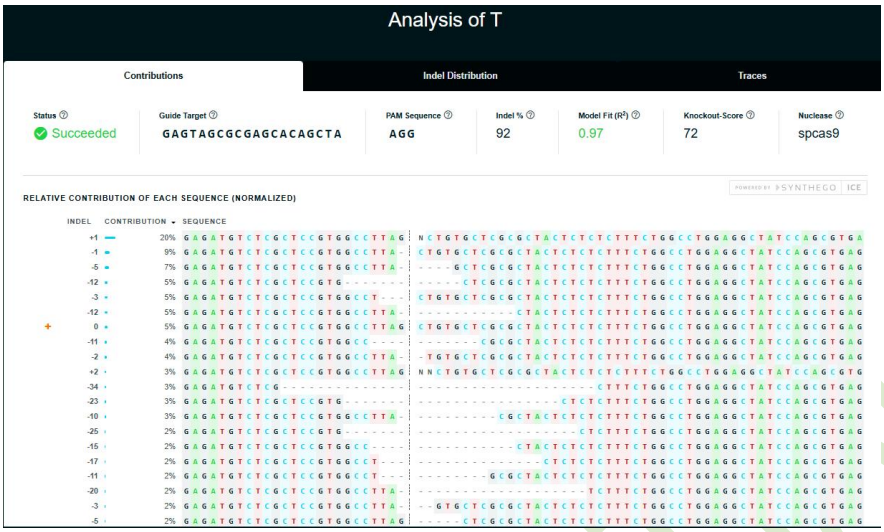

Figure 3. ICE Analysis of Polyclonal Editing in HEK293T Cells (Target: *B2M*)

Selected List of Successfully Edited Cell Lines

Cell Type	Editing Efficiency	Cell Type	Editing Efficiency	Cell Type	Editing Efficiency
Jurkat	97%	SK-OV-3	90%	MKN-45	71%
786-O	96%	HEK293	90%	SH-SY5Y	66%
U2OS	96%	NCI-H1299	90%	AsPC-1	64%
U251 MG	95%	HGC-27	89%	OCI-AML-2	64%
UM-UC-3	95%	KYSE-30	88%	HEPG2	61%
NCI-H1703	94%	NCI-H716	86%	NCI-H460	61%
HEC-1-B	94%	A549	85%	HT-29	61%
Hela	93%	U937	85%	PC-3	55%
HEK293T	92%	Caco-2	82%	SW579	52%
DMS273	92%	JAR	81%	THP-1	51%
U-87MG	92%	RD	79%	HEL	50%
A673	92%	NCI-H520	75%	CAL-33	48%
SNU-398	91%	OCI-AML3	74%	FaDu	44%
RKO	91%	A375	72%	NCI-H3122	42%

HUCC-T1	90%	K562	73%	KLE	42%
SNU-1	90%	MM.1S	73%	OE33	42%

FAQs

1. **How can high editing efficiency be achieved without selection?**

Answer: This kit has been validated across multiple cell lines. The RNP complex enters cells and begins gene editing within 4 hours post-transfection. Cas9 protein is degraded within 24–48 hours, allowing efficient and transient expression-driven editing without the need for antibiotic or fluorescent selection.

2. **Why does this kit cause minimal cellular damage?**

Answer: The kit employs advanced biomolecular transfection technology, offering significant advantages over traditional methods. Unlike chemical transfection, which may be cytotoxic, or electroporation, which can subject cells to physical stress, this approach ensures minimal damage while maintaining high efficiency.

3. **Can comparable editing efficiency be achieved in suspension cells?**

Answer: While suspension cells are generally more difficult to transfect, this kit performs exceptionally well in both adherent and suspension cell types. For example, in Jurkat cells, editing efficiency can reach up to 97% within 48 hours post-transfection, demonstrating the kit's outstanding performance and suitability for demanding suspension cell applications.

4. **What should I do if gene knockout fails using this kit?**

Answer: If gene knockout is unsuccessful when using this kit, EDITGENE will waive the cost of the kit. Moreover, the amount you paid can be fully credited toward EDITGENE's customized gene knockout services.

Precautions

1. This product is intended for laboratory research use only. Please strictly comply with all applicable laws, regulations, and ethical guidelines. Any consequences arising from improper use are the sole responsibility of the user, and EDITGENE Co., Ltd. assumes no liability.
2. Please follow the specified instructions for transportation, storage, and usage of the reagents. Avoid repeated freeze-thaw cycles unless absolutely necessary. EDITGENE Co., Ltd. is not responsible for any experimental failures resulting from improper storage or handling.