Flash-Pro CRISPR KO Kit (For Organoids / Stem Cells)

I. Basic Information

Cat. No.	Product Name	Size
EDKO-K05	Flash-Pro CRISPR KO Kit	50 μL
		100 μL

- **Product Overview**

The Flash-Pro CRISPR KO Kit achieves efficient and precise gene knockout by delivering pre-assembled complexes of delivery vectors, Cas9 protein, and sgRNA (RNP). This ready-to-use gene knockout kit is specially designed for research applications and includes EDITGENE's innovatively developed CRISPR RNP delivery vectors and Cas enzymes validated in thousands of experimental cases. The specially processed delivery vector-RNP complexes enable highly efficient genome editing in mammalian cells.

- **Storage Conditions and Shelf Life**

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

It is recommended to aliquot the product according to usage to avoid repeated freeze-thaw cycles.

- **Product Advantages**

High Editing Efficiency: Efficient delivery vectors and direct RNP delivery achieve gene knockout efficiencies up to 95%.

Short Workflow: The kit provides integrated delivery vector-RNP complexes, allowing DNA cleavage detection within 6 hours and complete gene knockout within 48 hours.

Broad Applicability: Suitable for most mammalian cells, including hard-to-transfect or limited-proliferation cells.

Easy Operation: No complex procedures or electroporation required; ready-to-use for efficient editing. The transient action mechanism eliminates the need for antibiotic or fluorescent selection, saving experimental time.

Low Risk: Minimal off-target effects; RNPs have a short intracellular half-life (a few hours), and Cas proteins are rapidly degraded, significantly reducing non-specific cleavage. There is no risk of random integration of exogenous DNA.

Low Cytotoxicity: The innovative biomolecule delivery vectors combined with RNP delivery minimize cellular damage.

- **Kit Components**

Cat. No.	Component	Size	Notes
EDKO-K05-50	Delivery Vector-RNP Complex	50 µL (sufficient for 3 × 24-well plates)	24-well plate, 15 µL per well
EDKO-K05-100	Delivery Vector-RNP Complex	100 µL (sufficient for 3 × 12-well plates)	12-well plate, 30 µL per well

***Note**: This product provides only the integrated delivery vector-RNP complex. Users only need to supply the sgRNA sequence.*

- **Optional Components**

Option	Notes
sgRNA Design	EDITGENE provides sgRNA sequence design services
Positive Control	Delivery vector-RNP complex targeting human B2M gene

II. Organoid Experimental Procedures

1. Organoid Culture and Seeding (24-Well Plate Example)

Culture organoids until healthy and actively growing. Digest and count cells, then seed 1.2×10^5 cells in 50 µL per well of a 24-well plate.

Note: Use well-growing organoids free from bacterial, fungal, or mycoplasma contamination. Digest organoids into 3-5 cell clusters. For organoids recently thawed from liquid nitrogen, passage at least twice before transfection.

2. Organoid Transfection

(1) Transfer the delivery vector-RNP complexes from -80 °C to 4 °C and thaw slowly. Add 15 µL per well slowly, supplement with complete medium to a total volume of 100 µL, mix gently, and centrifuge the 24-well plate at 32 °C, 500 g for 90 minutes.

(2) Collect organoid cells, centrifuge to separate cell pellets and supernatant. Embed the cell pellet for culture; use the supernatant with additional complete medium for maintenance.

(3) After 24 hours of transfection, replace with fresh complete medium and continue culture.

Notes: During transfection, the cell number is low and the procedure involves multiple steps, so take care to avoid losing cells during handling. If a plate centrifuge is not available, 2 mL Eppendorf tubes are recommended as an alternative, while 1.5 mL tubes are not advised.

3. Analysis of Transfected Cells

72 hours post-transfection, extract genomic DNA from transfected cells and amplify the target region using specific primers (amplicon includes the sgRNA target site).

Analyze gene editing efficiency using TIDE (https://tide.nki.nl/) or ICE (https://ice.synthego.com/#/, guide: https://www.synthego.com/guide/how-to-use-crispr/ice-analysis-guide).

III. Cell Experimental Procedures

1. Cell Culture and Seeding (24-Well Plate Example)

Culture cells until they are in a vigorous growth state. 24 hours before transfection, seed the cells into a 24-well plate. For adherent cells, seed so that the confluency at transfection is 50-60%. For suspension cells, seed 1.2×10^5-1.6×10^5 cells per well.

***Note**: Use healthy cells and ensure that they are free of bacterial, fungal, or mycoplasma contamination. For cells recently thawed from liquid nitrogen, passage at least twice before transfection.*

2. Cell Transfection

Thaw the delivery vector-RNP complexes slowly by transferring them from -80 °C to 4 °C. Add 10 μL per well slowly in multiple portions, and gently mix after addition.

For adherent cells: Ensure cells are healthy, evenly distributed, and at 50-60% confluency before adding the product directly.

For suspension cells: Ensure cells are healthy and gently disperse any cell clumps before adding the delivery vector-RNP complexes directly, so that the cells are evenly distributed.

3. Analysis of Transfected Cells

48 hours post-transfection, extract genomic DNA from the transfected cells and amplify the target region using specific primers (the amplicon should include the sgRNA target site).

Analyze gene editing efficiency using TIDE (https://tide.nki.nl/) or ICE (https://ice.synthego.com/#/, guide: https://www.synthego.com/guide/how-to-use-crispr/ice-analysis-guide).

IV. Results

- **Organoid Results**

Figure 1. Sanger sequencing alignment of mouse tongue organoids (specific gene).

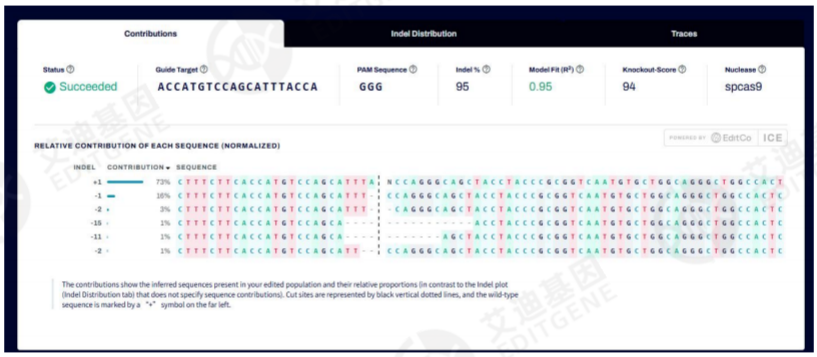

Figure 2. Multiclonal ICE analysis of mouse tongue organoids (specific gene).

- **Stem Cell Results**

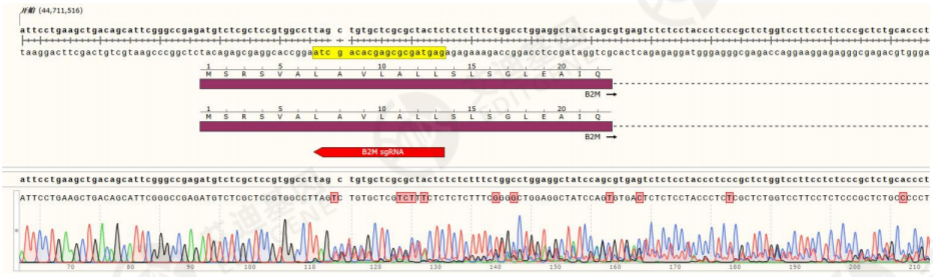

Figure 3. Sanger sequencing alignment of hIPSC-B4 cells (B2M).

Figure 4. Multiclonal ICE analysis of hIPSC-B4 cells (B2M).

V. Partial List of Successfully Edited Cells

Cell Type	Editing Efficiency	Cell Type	Editing Efficiency	Cell Type	Editing Efficiency
Raw264.7	100%	SNU-1	90%	H9	75%
CHO-K1	100%	HEK293	90%	SK-N-AS	75%
A9	99%	PANC-1	90%	OCI-Aml3	74%
Mouse tongue organoids	98%	SGC-7901	90%	VCaP	74%
JURKAT	97%	HMRSV5	90%	ARPE-19	74%
HK-2	97%	MDA-T32	90%	MM1S	73%
SN4741	97%	HGC-27	89%	K-562	73%
786-0	96%	U-87-MG	89%	A549	73%
U2OS	96%	Caki-1	89%	MKN45	71%
HUH-6	96%	LX-2	89%	HT-1080	71%
UM-UC-3	95%	3T3-L1	89%	SH-SY5Y	66%
U251	95%	KYSE-30	88%	MDA-MB-231	66%
hIPSC-B4	95%	AGS	88%	SNT-8	66%
IBMDM	94%	HLE-B3	88%	LO2	65%

HEC-1-B	94%	143B	87%	AsPC-1	64%
NCI-H1703	94%	MC38	87%	OCI-AML2	64%
SW13	94%	BV2	86%	NCI-H460	61%
NALM6 clone G5	94%	NCI-H716	86%	hepg2	61%
hela	93%	NCI-H1299	85%	HT-29	61%
Huh7	93%	SNU-449	84%	MB49	57%
DLD-1	92%	KMRC-1	84%	PC-3	52%
DMS-273	92%	SVG p12	84%	SW579	52%
HEK293T	92%	MOLM-13	84%	5637	52%
Hap1	92%	U-937	83%	THP-1	51%
A673	92%	KMS-12-PE	83%	HEL	50%
Ishikawa	92%	CACO-2	82%	CAL-33	48%
SNU-398	91%	CAL-27	82%	SK-N-SH	48%
RKO	91%	JAR	81%	Calu-3	47%
KYSE150	91%	NIH/3T3	81%	A375	46%
SK-BR-3	91%	MPC5	80%	FaDu	44%
2V6.11	91%	RD	79%	OE33	42%
MDA-T41	91%	NCTC929	79%	NCI-H3122	42%
ID8	91%	GBC-SD	78%	KLE	42%
HuCCT1	90%	HCT-116	77%	C2C12	42%
SK-OV-3	90%	NCI-H520	75%	OVCAR-3	40%

VI. FAQ

1. How can it be demonstrated that high editing efficiency is achieved without selection?

The product has been validated in multiple cell types. The RNP system enters cells and begins functioning within 4 hours post-transfection, and the Cas9 protein is degraded within 24-48 hours.

This transient, high-efficiency expression enables gene editing without the need for continuous selection.

2. Why does the product cause relatively low cellular damage?

The product utilizes advanced biomolecular transfection technology. Compared with the toxicity of traditional chemical transfection methods and the physical stress of electroporation, it shows significant advantages in preserving cell viability.

3. Can the same efficiency be achieved in suspension cells?

Transfecting suspension cells is generally more challenging. However, due to its superior performance, this product works efficiently not only in adherent cells but also in suspension cells. For example, in Jurkat cells, 48 hours post-transfection, editing efficiency can reach up to 97%, demonstrating the product's high efficiency in suspension cell transfection and meeting demanding requirements.

4. What should be done if gene knockout fails using the kit?

If gene knockout fails when using this kit, EDITGENE will not charge for the kit. Additionally, the fee you paid for the kit can be directly applied toward EDITGENE's gene knockout service, ensuring that your gene editing experiments proceed without concerns.

VII. Publishing Requirements

When using this product in publications, please acknowledge our company: Guangzhou Editgene Co. Ltd, China, CRISPR RNP KO kit (CAS：EDKO-K05). Or EDITGENE CO.LTD, CRISPR RNP KO kit (CAS：EDKO-K05), if used within U.S. or Europe territory.