Version: 01
Update: 11/02/2021

DATASHEET

GenCRISPR™ Cas9 v1.2

Cat. No.: Z03702-100; Z03702-500; Z03702-1
Size: 100 μg / 500 μg / 1 mg

Product Introduction

The GenCRISPR™ Cas9 v1.2 can be formed with the guide RNA into a ribonucleoprotien (RNP) complex. The use of an RNP complex to perform gene editing has been shown to reduce the challenges encountered with other CRISPR gene editing techniques such as viral and plasmid delivery. Challenges include off-target effects, cell viability and transcription/translational challenges.

GenCRISPR™ Cas9 v1.2 is a tag free nuclease produced by expression in an *E. coli* strain carrying a plasmid encoding the Cas9 gene from *Streptococcus pyogenes* with a biparticle nucleus localization signal (BPNLS) at N-terminal and a nucleoplasmin nucleus localization signal (nucleoplasmin NLS) at C-terminal. It has been reported that BPNLS and nucleoplasmin NLS are able to improve the gene editing efficiency.

Source: Recombinant Cas9 with a BPNLS at N-terminal and a nucleoplasmin NLS at C-terminal expressed by *E.coli*
Species: *Streptococcus pyogenes*
Tag: Tag-free
Molecular Weight: ~160 kDa
Concentration: 4 mg/ml
Active temperature: This Cas9 is active at 37°C.

Formulation: Supplied as a solution of 25 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 50% glycerol, pH 8.0.
Storage& Stability: This product remains stable for up to 12 months at -20°C. Avoid repeated freeze-thaw cycles
Application: gRNA-dependent double-stranded DNA cleavage

Quality Control Specifications

Assay	Specifications
Appearance	Clear, colorless liquid
Purity	≥ 90% as analyzed by SDS-PAGE
Concentration by A280	4 mg/ml±10%
Bioactivity (*in vitro*)	≥ 90%
Residual DNase	Non-specific DNase activity
Residual RNase	Non-specific RNase activity

Endotoxin Level	≤ 100 EU/mg as analyzed by gel clotting method

Reagents Supplied:

Components	Amount	Storage
GenCRISPR™ Cas9 v1.2	100 μg; 500 μg; 1 mg	-20 °C

Reagents required but not provided:

a. Nuclease-free water
b. sgRNA containing the targeting sequence in the region of interest
   - ❖ GenCRISPR sgRNA Screening Kit (L00689)
   - ❖ GenCRISPR sgRNA Synthesis Kit (L00694)
   - ❖ CRISPR Synthetic Guide RNA Services (gene@genscript.com)
c. Proteinase K for *in vitro* digestion of DNA
d. Substrate DNA containing the target sequence for *in vitro* digestion of DNA
   - ❖ DNA Synthesis Services (gene@genscript.com)
e. HDR donor template containing the target sequence for knock-in application
   - ❖ Single-stranded DNA Synthesis Services (gene@genscript.com)
   - ❖ Double-strand DNA Synthesis Services (gene@genscript.com)
f. Cas9 transfection reagents by electroporation or lipofection
g. GenCRISPR Mutation Detection Kit (L00688)
h. GenCRISPR T7 Endonuclease I (Z03396)

Protocols for multiple applications

1. Operation procedures for *In vitro* digestion of DNA

1.1 Reagents preparations

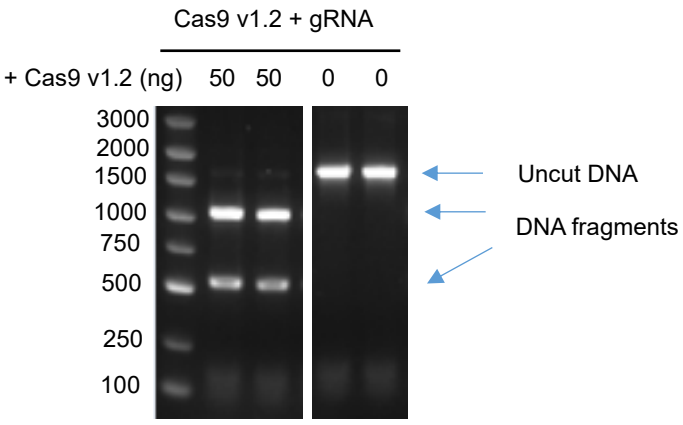
a. Prepare the specific sgRNA, we recommend GenCRISPR sgRNA Synthesis Kit (L00694) for the sgRNA *in vitro* transcription or CRISPR Synthetic Guide RNA Services (gene@genscript.com) for sgRNA synthesis.
b. Prepare the specific substrate DNA, we recommend GenScript DNA Synthesis Service (gene@genscript.com) for the applications, or customers can also apply PCR method to obtain substrate DNA (**Note:** the PCR product needs to be purified prior to further assay).
c. Prepare the sgRNA work solution at an appropriate concentration (eg., 50 ng/μl) by diluting the stock solution with nuclease-free water on ice.
d. Prepare the substrate DNA work solution at an appropriate concentration (eg., 80 ng/μl) by diluting the stock solution with nuclease-free water on ice.
e. 10 × Reaction Buffer (If needed, please contact us for free stock buffer).
f. The reaction volume is typically 20 μl used in our laboratory, but can be adjusted according to customers' specific applications.

1.2 Assay procedures

Assemble the reaction in a nuclease-free microfuge tube or PCR strip tube at room temperature in a Clean Bench as the following order:

Components	Volume
10× Reaction Buffer	2 µl
160 ng Substrate DNA	2 µl (80 ng/µl)
100 ng sgRNA	2 µl (50 ng/µl)
50 ng GenCRISPR™ Cas9 v1.2 (Z03702)	2 µl (25 ng/µl)
Nuclease-free water	12 µl
Reaction volume	20 µl
Mix thoroughly, incubate for 1 hour at 37 ºC;	
Add 1 µl Proteinase K (20 µg/µl), incubate for 30 min at 55 ºC;	
After incubation, determine the digestion efficiency by agarose gel electrophoresis.	

1.3 Example of Case for in vitro DNA digestion



A 20 µl reaction in 1 × Cas9 Nuclease Reaction Buffer containing linearized plasmid, gRNA, and Cas9 for 1 hour at 37 °C results in a digestion efficiency of linearized plasmid higher than 90%, as determined by agarose gel electrophoresis.

2. Operation procedures for gene knock out in cell lines

2.1 Reagents preparations

a. Prepare the specific sgRNA, we strongly recommend GenScript sgRNA Synthesis Service (gene@genscript.com) for the applications.

b. Prepare the sgRNA work solution at an appropriate concentration (eg., 125 pmol/µl) by diluting the stock solution with nuclease-free water on ice.

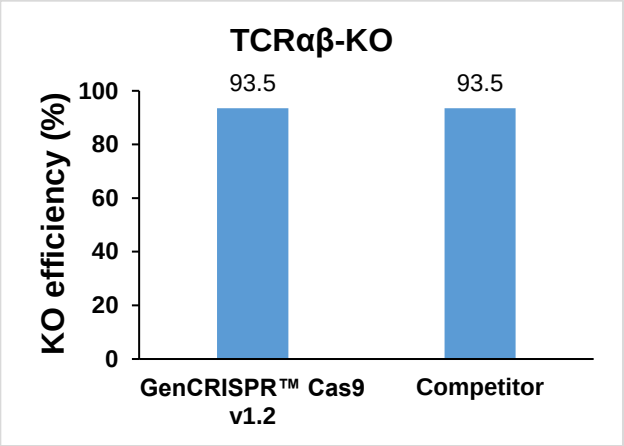
c. Transfer your cells to a new complete media according to experimental groups 1-2 days before transfection. Adherent cells should be around 80% confluent on the day of transfection.

d. Prepare the transfection reagents for Cas9 transfection. Electroporation is typically used in our laboratory to get higher transfection efficiency for various cell types. Customers can also apply lipofection method for specific assays referring to suppliers' protocols.

2.2 Assay procedures

<table>
<tr><th>Steps</th><th>Operations</th></tr>
<tr><td>Step 1: Prepare cell culture media</td><td>a. On the day of transfection, add cell type specific growth media to the plate and pre-warm it at 37 °C. (For example, transfer 500µl complete media for T cell culture into each well of a 24-well-plate, incubate at 37 °C to pre-warm.)
b. Set the electroporation program prior to transfection.</td></tr>
<tr><td>Step 2: Prepare the RNP complex</td><td>a. Mix the GenCRISPR™ Cas9 v1.2 and sgRNA at a 1: 3 molar ratio with the electroporation buffer in a nuclease-free microfuge tube or PCR strip tube referring to the following table:
<table>
<tr><th>Reagent</th><th>Amount</th></tr>
<tr><td>GenCRISPR™ Cas9 v1.2 (Z03702)</td><td>7.5 pmol (1200 ng)</td></tr>
<tr><td>sgRNA</td><td>22.5 pmol (682 ng)</td></tr>
<tr><td>Total reaction volume</td><td>10 µl</td></tr>
</table>
b. Incubate the mix solution at 37 °C for 15 min to assemble the RNP complex (~ 7.5 pmol).</td></tr>
<tr><td>Step 3: Electroporate using cell type specific transfection reagents</td><td>a. Centrifuge the cells for 5 min at 800 rpm at room temperature, remove the cell culture supernatant as far as possible.
b. Wash the cells with 1 × PBS, centrifuge the cells for 5 min at 800 rpm at room temperature, remove the 1 × PBS as far as possible.
c. Aspirate 8 µl electroporation buffer to suspense 0.5 × 10^6 cells, mix thoroughly.
d. Aspirate 8 µl cell suspension (from step 3c) to the RNP complex (from step 2), mix thoroughly.
e. Using the cell type specific electroporation reagents (from other suppliers) to proceed the transfection referring to the suppliers' protocols.
Note: 1) Avoid creating bubbles when pipetting and mixing reagents;
2) Process the transfection after mixing the RNP complex and cells as soon as possible.
f. After the electroporation, transfer the cell mixture immediately to the pre-warmed complete media (from Step 1a). Culture the cells at an incubator (37 ºC, 5% CO_2) for 24 hours - 48 hours.</td></tr>
<tr><td>Step 4: Gene editing analysis and perform downstream applications</td><td>a. It's recommended to apply NGS or Sanger Sequencing (seq@genscript.com) to determine the indel efficiency. Or use the GenCRISPR Mutation Detection Kit (L00688) to verify the gene editing efficiency and select the highest editing efficiency clone.
b. Proceed the downstream applications according to customers' own design.</td></tr>
</table>

2.3 Example of Case for gene knock-out in cell lines



Human Jurkat cells were cultured for the test. The cells were transfected with GenCRISPR™ Cas9 v1.2 (Z03702)+ sgRNA (synthesized from GenScript gene@genscript.com) for human TCRαβ gene knock out by electroporation. After transfection and cell culture, measure the gene editing efficiency. GenCRISPR™ Cas9 v1.2 shows a much high editing efficiency.

3. Operation procedures for gene knock-in in cell lines

3.1 Reagents preparations

a. Prepare the specific sgRNA, we strongly recommend GenScript sgRNA Synthesis Service (gene@genscript.com) for the applications.

b. Prepare the sgRNA work solution at an appropriate concentration (eg., 125 pmol/μl) by diluting the stock solution with nuclease-free water on ice.

c. Prepare the specific HDR donor template, we strongly recommend GenScript DNA Synthesis Service (gene@genscript.com) for the applications.

d. Transfer your cells to a new complete media according to experimental groups 1-2 days before transfection. Adherent cells should be around 80% confluent on the day of transfection.

e. Prepare the transfection reagents for Cas9 transfection. Electroporation is typically used in our laboratory to get higher transfection efficiency for various cell types. Customers can also apply lipofection method for specific assays referring to suppliers' protocols.

3.2 Assay procedures

Steps	Operations
Step 1: Prepare cell culture media	a. On the day of transfection, add cell type specific growth media to the plate and pre-warm it at 37 °C. (For example, transfer 240 μl complete media for T cell culture into each well of a 48-well-plate, incubate at 37 °C to pre-warm.) b. Set the electroporation program prior to transfection.

<table>
<tr><td>Step 2: Prepare the RNP complex</td><td>a. Mix the GenCRISPR™ Cas9 v1.2 and gRNA at a 1: 2 molar ratio with the electroporation buffer in a nuclease-free microfuge tube or PCR strip tube referring to the following table:
<table><tr><th>Reagent</th><th>Amount</th></tr><tr><td>GenCRISPR™ Cas9 v1.2 (Z03702)</td><td>25 pmol (4000 ng)</td></tr><tr><td>sgRNA</td><td>50 pmol (1515 ng)</td></tr><tr><td>Total reaction volume</td><td>20 µl</td></tr></table>b. Incubate the mix solution at 37 °C for 15 min to assemble the RNP complex (~25 pmol).</td></tr>
<tr><td>Step 3: Electroporate using cell type specific transfection reagents</td><td>a. Centrifuge the cells for 10 min at 300 g at room temperature, remove the cell culture supernatant as far as possible.
b. Wash the cells with 1 × PBS, centrifuge the cells for 10 min at 300 g at room temperature, remove the 1 × PBS as far as possible.
c. Aspirate 20 µl electroporation buffer to suspense 1.0 × 10^6 cells, mix thoroughly.
d. Aspirate 20 µl cell suspension (from step 3c) and 1-3 µg HDR donor template to the RNP complex (from step 2), mix thoroughly.
e. Using the cell type specific electroporation reagents (from other suppliers) to proceed the transfection referring to the suppliers' protocols.
Note: 1) Avoid creating bubbles when pipetting and mixing reagents;
2) The optimal amount of HDR donor used in the reaction should be determined by customers' own experiences.
3) Process the transfection after mixing the RNP complex and cells as soon as possible.
f. After the electroporation, transfer the cell mixture immediately to the pre-warmed complete media (from Step 1a). Culture the cells at an incubator (37 ºC, 5% CO_2) for 24 hours - 48 hours.</td></tr>
<tr><td>Step 4: Gene editing analysis and perform downstream applications</td><td>a. It's recommended to apply NGS or Sanger Sequencing (seq@genscript.com) to determine the indel efficiency. Or use the GenCRISPR Mutation Detection Kit (L00688) to verify the gene editing efficiency and select the highest editing efficiency clone.
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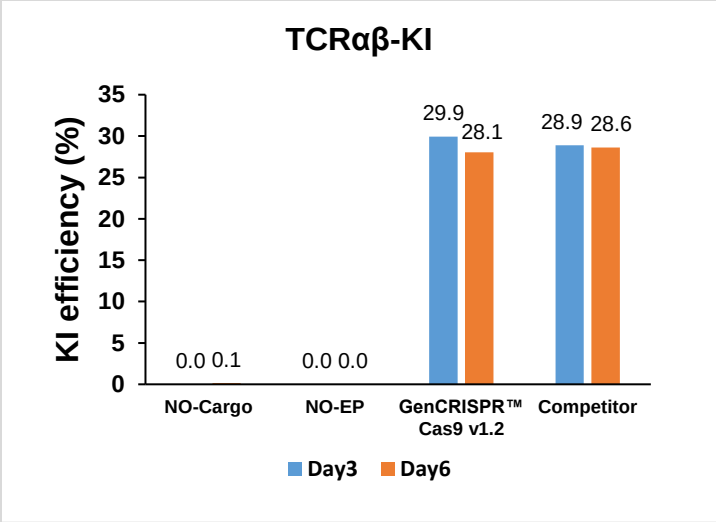
3.3 Example of Case for gene knock-in in cell lines



Human T cells were cultured for the test. The cells were transfected with GenCRISPR™ Cas9 v1.2 (Z03702)+ sgRNA (synthesized from GenScript gene@genscript.com) + dsDNA template (synthesized from GenScript gene@genscript.com) for knock in test at TCRαβ site by electroporation. After transfection and cell culture, measure the editing efficiency.

Key Features

High gene editing efficiencies: Consistent high editing efficiency in *in-vitro* and *in-vivo*.
Tag-free: Amino acid is free from additional tagging amino acid.
DNA-free: No external DNA added to the system.

References:

1. Liu, Xinyi, et al. "Improving editing efficiency for the sequences with NGH PAM using xCas9-derived base editors." Molecular Therapy-Nucleic Acids 17 (2019): 626-635.
2. Pollard, Victoria W., et al. "A novel receptor-mediated nuclear protein import pathway." *Cell* 86.6 (1996): 985-994.
3. Koblan, Luke W., et al. "Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction." *Nature biotechnology* 36.9 (2018): 843-846.

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