

Cas9 circRNA

Cat. No.: MR209

01/ Description

The Cas protein (Cas9), discovered in *Streptococcus pyogenes*, is one of the most widely used Cas proteins. Cas9 functions as a DNA nuclease within the CRISPR genome-editing system. Guided by specific sgRNA, Cas9 targets designated sites in the genome and cleaves double-stranded DNA (dsDNA), resulting in double-strand breaks (DSBs).

Cas9 mRNA encodes the Cas9 protein, which is equipped with nuclear localization signals (NLS) at both the N- and C-termini to facilitate nuclear entry, thereby enhancing DNA cleavage efficiency. Additionally, the C-terminal Flag epitope tag aids in the detection, isolation, and purification of Cas9 protein.

This product is a mature circRNA synthesized in vitro using Circular RNA Synthesis Kit (PIE I) (Novoprotein, Catalog No.: E336), followed by Type I intron self-splicing. The unique covalently closed circular structure of circRNA prevents degradation by exonucleases, providing enhanced biological stability. Therefore, it is an ideal reagent for studying RNA transfection and protein expression using various analytical methods.

02/ Features

1. Compared with linear mRNA, circRNA has greater resistance to RNase degradation
2. Longer half-life
3. Stable transfection efficiency

03/ Basic Information

Gene Information	Uniprot: Q99ZW2
Species	<i>Streptococcus pyogenes</i>
Amino Acid Sequence	View Sequence
mRNA Sequence Length	5076nt
Theoretical Molecular Weight	1,637,446.2 g/mol
Tag	NLS, C-flag
Concentration	1mg/ml

04/ Applications

Cas9 circRNA enables the transient expression of Cas9 protein within cells, which, guided by the crRNA and tracrRNA complex, cleaves the target DNA sequence. It is well-suited for gene editing research in mammalian cells.

05/ Storage Temperature

Storage at -20°C ± 5°C.

06/ Packaging

Product Component	MR209-U100	MR209-M001
Cas9 circRNA (1mg/ml)	100µg	1 mg

07/ Operating Instructions (Intracellular Editing)

(1) Required Materials (to be prepared by the user)

- a. Target cells for editing
- b. sgRNA
- c. Transfection reagents
- d. Other relevant reagents

(2) Transfection

To achieve optimal editing efficiency, the ratio of circRNA to sgRNA can be optimized based on the target cell population. For initial experiments, consider starting with the following recommended conditions:

Cas9 circRNA and sgRNA should be mixed at a 1:1 mass ratio. For a 24-well cell culture plate, it is recommended to use 4 µg of circRNA per well.

(3) Electroporation in 293T Cells for Gene Knockout Efficiency Evaluation (*Applicable for in vivo gene target screening and cell line construction*)

1. Cell Preparation:

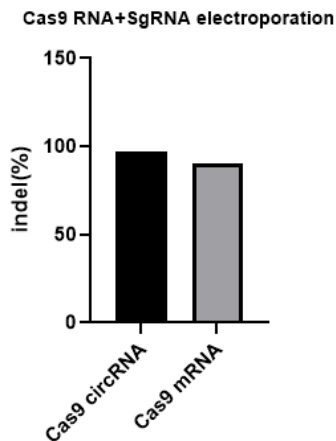
- a) Preparation: Place trypsin at room temperature for 1–2 hours and preheat the basal medium and PBS in a 37°C water bath for 1–2 hours.
- b) Observation: Under a microscope, observe the cells in the culture flask to confirm they are healthy and at 70–90% confluence.
- c) Digestion: Discard the medium in the culture flask, rinse once with PBS (3 ml for T75 flasks, 1 ml for T25 flasks) once, discard the PBS, and add trypsin (3 ml for T75 flasks, 1 ml for T25 flasks). Incubate in a 37°C incubator for digestion, which usually takes 1–10 minutes depending on the cell line.
- d) Terminate Digestion: Add basal medium (6 ml for T75 flasks, 3 ml for T25 flasks) to stop digestion. Gently pipette to form a single-cell suspension, then transfer about 100–300 µl of cell suspension to an Eppendorf tube.
- e) Cell Counting: Pipette 20 µl of cell suspension into an Eppendorf tube, add 20 µl of 0.2% trypan blue, mix well, and immediately take 20 µl for loading into the sample slot on a counting chamber. Place it in a cell counter, examining three fields of view.
- f) Prepare 1×10^6 cells per sample. Centrifuge at 300 g for 5 min at room temperature. Discard supernatant, centrifuge again at 100 g for 2 min, and carefully remove residual liquid using a 200 µL pipette.

2. circRNA Substances Transfection:

- a) sgRNA preparation: dissolve sgRNA powder in nuclease-free water to 2–4 µg/µL.
- b) RNA mixture preparation: prepare a Cas9 circRNA + sgRNA mixture in a 1.5 mL microcentrifuge tube and mix gently.
- c) Add cells: add electroporation solution and cell suspension to the RNA mixture and mix gently, avoiding bubble formation.
- d) Transfer to cuvette: transfer the entire mixture into an electroporation cuvette ($\leq 30 \mu\text{L}$ total volume). Avoid bubbles throughout the process.
- e) Electroporation: using a Lonza electroporation system:
 - Select X module.
 - Insert electroporation cuvette.
 - Select 293T cells.
 - Choose program DS150.

Start the electroporation. After completion, remove the cuvette and turn off the instrument.

- f) Post-electroporation culture: add 100 μ L 1 $\times$ antibiotic-containing complete medium to each well and incubate 15 min in the incubator. Remove the supernatant, resuspend cells in 120 μ L culture medium, transfer to a 24-well plate, and add medium to a final volume of 1 mL. Incubate under standard culture conditions.
3. Genomic DNA Extraction and Sequencing Analysis:
 - a) 24 h post-transfection: observe cell morphology under a microscope and capture images.
 - b) 72 h post-transfection: observe cell status and collect cells for genomic DNA extraction. Amplify the target region by PCR and perform sequencing.
 - c) Gene editing analysis: perform Sanger sequencing and analyze editing efficiency using SnapGene and TIDE (TIDE: Tracking of Indels by Decomposition).
4. Result:



No.	mRNA	sgRNA	Editing Efficiency (Tide%)
1	Cas9 circRNA	TRAC	97.3
2	Cas9 mRNA	TRAC	90.5

After electroporation of Cas9 circRNA + target sgRNA into 293T cells, TIDE analysis showed that the editing efficiency at the TRAC locus exceeded 90%.

08/ Amino Acid Sequence

MPKKKRKVDKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNR
 ICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
 RGHFLIEGDLNPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPN
 FKSNFLAEDAQLQSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASMIKRYDEHHQDITLLK
 ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAIL
 RRQEDFYFPLKDNREKIEKILTFRIPIYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASQSFIERMTNFDKNLPNEKVLP
 KHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT
 YHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTI
 LDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMA
 RENQTTQKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLK
 DDSIDNVKVLTRSDKNRGKSDNPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVA
 QILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGTAIKKYPKLESEFVYGDYKVV
 DVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVKKTEVQT

GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGSKKLKSVKELLGITIMERSSSFENPIDFLEAKGY
KEVKKDLIIKLPKYSLFELNGRKRMLASAGELQKGNELALPSKYVNFYLLASHYEKLKGSPEQNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRID
LSQLGGDGGSGPPKKKRKVASGSKRPAATKKAGQAKKKKDYKDDDDK

09/ Related Product

Cat. No.	Product Name	Cat. No.	Product Name
GMP-M062	Vaccinia Capping System, GMP Grade	GMP-E121	T7 RNA Polymerase, GMP Grade
GMP-M072	mRNA Cap 2'-O-Methyltransferase, GMP	GMP-E125	RNase Inhibitor, GMP Grade
GMP-M012	E. coli Poly(A) Polymerase, GMP Grade	GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade
GMP-E122	T7 RNA Polymerase 2.0, GMP Grade	MR010	eGFP mRNA (N1-Me-Pseudo UTP)
MR008	eGFP mRNA	MR302	Firefly Luciferase saRNA
MR009	Luciferase mRNA	MR301	eGFP saRNA
MR011	Firefly Luciferase mRNA (N1-Me-Pseudo UTP)	MR204	tdTomato mRNA (N1-Me-Pseudo UTP)
MR105	mCherry mRNA (N1-Me-Pseudo UTP)	MR019	Cas9 mRNA (N1-Me-Pseudo UTP)
MR015	OVA mRNA (N1-Me-Pseudo UTP)	MR016	hEPO mRNA (N1-Me-Pseudo UTP)