GenCrispr Cas9 Nuclease

Cat. No.: Z03386 Version 2018-07-16

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I Description

GenCrispr Cas9 Nuclease is the recombinant *Streptococcus pyogenes* Cas9 (wt) protein purified from *E. coli* that can be used for genome editing by inducing site-specific double stranded breaks in double stranded DNA. Cas9 protein forms a very stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component of the CRISPR/Cas9 system. The RNP complex recognizes the target site by matching gRNA with the genomic DNA sequence and produces DNA breaks within 3 bases from the NGG PAM (Protospacer Adjacent Motif). With GenCrispr Cas9 nuclease, customers can screen for highly efficient gRNA in vitro using DNA cleavage assays. The high purity Cas9 protein can also be used for antibody production.
Product Source: GenCrispr Cas9 Nuclease is produced by expression in an *E. coli* strain carrying a plasmid encoding the Cas9 gene from *Streptococcus pyogenes* without nuclear localization signal (NLS).

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II Contents

Contents	Quantity	Catalog No.	Components/Concentration
GenCrispr Cas9	10 µg	Z03386-10	0.2 mg/mL
	50 µg	Z03386-50	0.2 mg/mL
10X Reaction Buffer	1.5 mL		200 mM HEPES, 1 M NaCl, 50 mM $MgCl_2$, 1 mM EDTA, pH 6.5 at 25°C

III Key Features

- **High Protein Purity:** GenCrispr Cas9 Nuclease is > 95% pure as determined by SDS-PAGE with Coomassie Blue detection.
- **Non-specific DNase Activity:** A 20 µL reaction in Cas9 reaction buffer containing 100 ng linearized pUC57 plasmid and 0.1 µg of GenCrispr Cas9, incubated for 16 h at 37°C. No DNA degradation is determined by agarose gel electrophoresis.
- **Non-specific RNase Activity:** A 10 µL reaction in Cas9 reaction buffer containing 1800 ng total RNA and 0.1 µg of GenCrispr Cas9 incubated for 2h at 37°C. No RNA degradation as determined by agarose gel electrophoresis.
- **High Bioactivity:** 20 nM GenCrispr Cas9 incubated for 1 hour at 37°C result in 90% digestion of the substrate DNA as determined by agarose gel electrophoresis.

IV Storage

GenCrispr Cas9 is supplied with 1x storage buffer (10 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol pH 7.4 at 25°C). The recommended storage temperature is -20°C.

V Diluent Compatibility

Diluent Buffer: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 at 25°C).

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VI Activity test

Cas9 site-specific digestion:

GenScript used *in vitro* digestion of a linearized plasmid to determine the activity of the Cas9 nuclease. It is a sensitive assay for GenCrispr Cas9 quality control. The linearized plasmid containing the target site:

(CATCATTGGAAAACGTTCTT)

can be digested with gRNA:

(CAUCAUUGGAAAACGUUCUUGUUUUAGAGCUAGAAAU
AGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAA
AAAGUGGCACCGAGUCGGUGCUUUUUUUU)

and GenCrispr Cas9. Two cleavage DNA fragments (812 bp and 1898 bp) are determined by agarose gel electrophoresis. A 20 µL reaction in 1xCas9 Nuclease Reaction Buffer containing 160 ng linearized plasmid, 40 nM gRNA and 20 nM GenCrispr Cas9 for 2 hour at 37°C results in 90% digestion of linearized plasmid as determined by agarose gel electrophoresis.

VII References

1. Jinek et al. A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. (2012) Science 337 (6096) 816-821 (2012).
2. Larson, M. H., et al. CRISPR interference (CRISPRi) for sequence-specific control of gene expression.Nature Protocols. 8, (11), 2180-2196 (2013).
3. Ran, F. A., et al. Genome engineering using the CRISPR-Cas9 system. Nature Protocols. 8, (11), 2281-2308 (2013).

VIII Notes

1. This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.
2. 1000 nM is equal to 160 ng/ul.

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IX Ordering Information

Product Name	Cat. No.
GenCrispr Cas9-C-NLS Nuclease	Z03385
GenCrispr Cas9 Nuclease	Z03386
GenCrispr Cas9-N-NLS Nuclease	Z03388
GenCrispr NLS-Cas9-NLS Nuclease	Z03389
GenCrispr NLS-Cas9-D10A Nickase	Z03390
GenCrispr NLS-Cas9-EGFP Nuclease	Z03393
GenCrispr T7 Endonuclease I	Z03396
GenCrispr Mutation Detection Kit	L00688
GenCrispr sgRNA Screening Kit	L00689
High-Efficiency gRNA-Cas9-GFP Plasmid (linear) Assembly Kit	L00690
High-Efficiency gRNA-Cas9-Puro Plasmid (linear) Assembly Kit	L00691
High-Efficiency gRNA-Cas9-GFP Plasmid Assembly Kit	L00692
High-Efficiency gRNA-Cas9-Puro Plasmid Assembly Kit	L00693

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