GenCrispr Cas9 Nuclease
Cat. No. Z03386 **Version 01202016**

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).

II. KIT CONTENTS

Kit Contents	Catalog No.	Quantity	Components/Concentration
GenCrispr Cas9 Nuclease	Z03386-10	10 ug (50 ul)	0.2 mg/ml
	Z03386-50	50 ug (50 µl × 5)	0.2 mg/ml
10X Reaction Buffer		1.5 ml	200 mM HEPES, 1M NaCl, 50 mM $MgCl_2$, 1 mM EDTA, pH 6.5 at 25°C

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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).

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:

(CAUCAUUGGAAAACGUUCUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCC GUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUUU)

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 hours 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).

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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/μl.

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