

Version: 01
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DATASHEET

GenCRISPR™ Ultra eSpCas9-2NLS-Research

Cat. No.: Z03622-0.5; Z03622-1; Z03622-5

Size: 0.5 mg / 1 mg / 5 mg

Product Introduction

The GenCRISPR™ Ultra eSpCas9 product line provides customers with a selection of research use, basic GMP and GMP compliant Cas9 nucleases. The Cas9 protein can be formed with the guide RNA into a ribonucleoprotein (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™ Ultra eSpCas9-2NLS is a mutant form of Cas9 nuclease and is produced by expression in an *E. coli* strain carrying a plasmid encoding the eSpCas9 gene from *Streptococcus pyogenes* with double-ended nuclear localization signal (NLS). GenCRISPR™ Ultra eSpCas9-2NLS delivers higher fidelity and less off-target activity than wild-type SpCas9 nuclease.

Source: Recombinant Cas9 with double-ended NLS expressed by *E. coli*

Species: *S. pyogenes*

Tag: Tag-free

Molecular Weight: ~160 kDa

Concentration: 10 mg/mL

Active temperature: This eSpCas9 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 at 25°C.

Storage & Stability: This product remains stable up to 12 months at -20°C. Avoid repeated freeze-thaw cycles.

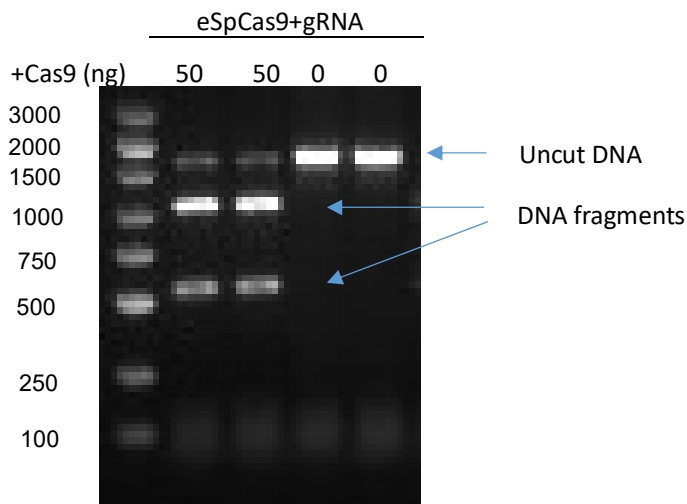
Application: gRNA-dependent double-stranded DNA cleavage

Quality Control Specifications

Assay	Specifications
Appearance	Clear, colorless liquid
Purity	≥ 95% as analyzed by SDS-PAGE ≥ 90% as analyzed by SEC-HPLC
Concentration by A280	10 mg/ml ± 1 mg/ml
Bioactivity (<i>in vitro</i>)	≥ 90%
Residual DNase	Non-specific DNase activity

Residual RNase	Non-specific RNase activity
Endotoxin Level	≤ 10 EU/mg as analyzed by gel clotting method

Data images:



A 20 μ l reaction in 1 \times Cas9 Nuclease Reaction Buffer containing 160 ng linearized plasmid, 100 ng gRNA, and 50 ng GenCRISPR™ Ultra eSpCas9-2NLS for 2 hours at 37°C results in a digestion efficiency of linearized plasmid higher than 90%, as determined by agarose gel electrophoresis.

Key features:

High knockout efficiencies: Consistent high performance in *in-vitro* plasmid cleavage test.

Tag-free: No extra tag amino acid

Animal-free: Plant-based culture media, Enzyme-free chromatographic purification.

DNA-free: No external DNA added to the system.

References:

1. Slaymaker, Ian M., et al. "Rationally engineered Cas9 nucleases with improved specificity." *Science* 351.6268 (2016): 84-88.2.
2. Fan, Rong, et al. "Shortening the sgRNA-DNA interface enables SpCas9 and eSpCas9 (1.1) to nick the target DNA strand." *Science China Life Sciences* 63.11 (2020): 1619-1630.
3. Chen, Janice S., et al. "Enhanced proofreading governs CRISPR–Cas9 targeting accuracy." *Nature* 550.7676 (2017): 407-410.
4. Popack, Dovber. *Increasing the accuracy of CRISPR/Cas9 gene editing for introns 44 and 55 of the DMD gene by using high fidelity Cas9 nucleases*. Diss. UCLA, 2021.

Related Products

Construct Design	Cat. No.	Product Name	Size
NLS-eSpCas9-NLS	Z03624	GenCRISPR™ Ultra eSpCas9-2NLS-basic GMP	1 mg / 2.5 mg / 5 mg
	Z03625	GenCRISPR™ Ultra eSpCas9-2NLS-GMP	2.5 mg / 5 mg
NLS-eSpCas9	Z03692	GenCRISPR™ Ultra NLS-eSpCas9-Research	0.5 mg / 1 mg / 5 mg
	Z03693	GenCRISPR™ Ultra NLS-eSpCas9-basic GMP	1 mg / 2.5 mg / 5 mg
	Z03694	GenCRISPR™ Ultra NLS-eSpCas9-GMP	2.5 mg / 5 mg

For laboratory research use only. Direct human use, including taking orally and injection and clinical use are forbidden.