GenCrispr NLS-Cas9-EGFP Nuclease
Cat. No. Z03393 **Version 08032016**

I DESCRIPTION

GenCrispr NLS-Cas9-EGFP is a fusion protein developed by GenScript. It contains a nuclear localization signal (NLS) on its N terminal end, and an EGFP and a 6x (His) sequence on the C terminal end. Cas9 nuclease is an RNA-guided endonuclease that can catalyze cleavage of double stranded DNA. This kind of targeted nuclease is a powerful tool for genome editing with high precision. Cas9 protein forms a highly stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component of the CRISPR/Cas9 system. When Cas9 is expressed with an NLS sequence, the Cas9 RNP complex can localize to the nucleus immediately upon entering the cell. There is no requirement for *in vivo* transcription or translation, which improves the efficiency of this method over plasmid-based systems. Additionally, the Cas9 RNP complex is rapidly cleared from the cells minimizing the chance of off-target cleavage when compared to other systems (Kim, et al. 2014). The EGFP tag can be used as a reporter for tracking or sorting transfected cells, which enables enrichment of cell populations for desired genome edits via fluorescence activated cell sorting (FACS). It significantly reduces the labor and cost associated with single cell cloning and genotyping in genome editing applications.

Product Source: GenCrispr NLS-Cas9-EGFP is produced by expression from an *E. coli* strain.

II KIT CONTENTS

Kit Contents	Quantity	Catalog No.	Components/Concentration
GenCrispr NLS-Cas9-EGFP Nuclease	50 µg	Z03393-50	1 mg/ml
	100 µg	Z03393-100	3 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

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III Key Features

- **DNA-free: no external DNA added to system**
- **High cleavage efficiency: NLS ensures the efficient entry of the Cas9 protein into nuclei**
- **Low off target effects: transient expression of Cas9 nuclease improves specificity of cleavage**
- **Time-saving: no need for transcription and translation**
- **Reduced labor: enrich cell populations for desired genome edits via EGFP-based FACS. The C-terminal His-tag increases the choice of detection methods for the fusion protein.**

IV Quality Control Analysis

➢ **High Protein purity:** GenCrispr Cas9 is > 95% pure as determined by SDS-PAGE using Coomassie Blue detection.

➢ **Low Endotoxin:** Endotoxin level is <0.1eu/μg test by gel-clot method: limit test.

➢ **Non-specific DNase activity:** A 20 μl reaction in Cas9 reaction buffer containing 100 ng linearized pUC57 plasmid and 0.1 μg 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 2 h 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 over 90% digestion of the substrate DNA as determined by agarose gel electrophoresis.

V Application

1. Screening for highly efficient and specific targeting gRNAs by *in vitro* DNA cleavage.
2. *In vivo* gene editing when combined with a specific gRNA by electroporation or injection.
3. Enrichment of cell populations for desired genome edits via EGFP-based FACS.

VI STORAGE

GenCrispr NLS-Cas9-EGFP is supplied with 1X storage buffer (10 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol pH7.4 at 25 °C) and recommended to be stored at -20 °C.

VII Diluent Compatibility

Diluent Buffer B: 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).

VIII Activity test

NLS-Cas9-EGFP 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 NLS-Cas9-EGFP quality control. The linearized plasmid containing the target site: (CATCATTGGAAAACGTTCTT) can be digested with gRNA:

(CAUCAUUGGAAAACGUUCUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUUU)

and GenCrispr NLS-Cas9-EGFP. Two cleavage DNA fragments (812 bp and 1898 bp) are determined by agarose gel electrophoresis. A 20 μl reaction in 1 x Cas9 Nuclease Reaction Buffer containing 160 ng

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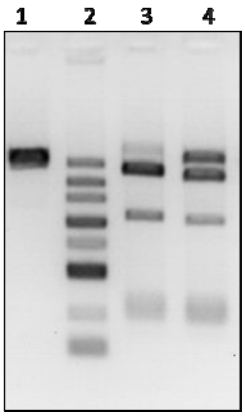
linearized plasmid, 40 nM gRNA and 20 nM GenCrispr NLS-Cas9-EGFP for 2 hour at 37 °C results in over 90% digestion of linearized plasmid as determined by agarose gel electrophoresis.



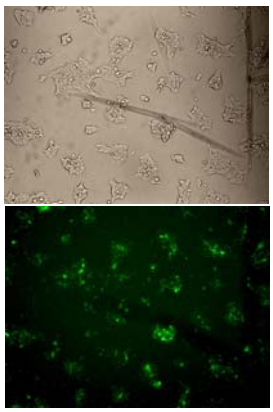
In vitro DNA cleavage assay with GenCrispr NLS-Cas9-EGFP nuclease

Reactions were set up according to recommended conditions, and cleavage products were resolved on a 1% agarose gel. Input DNA is EcoR V-linearized pUC57 plasmid DNA. Lane 1, DNA + gRNA; lane 2, marker; lane 3 and 4, DNA + gRNA + NLS-Cas9-EGFP 100 ng (lane 3) or 50 ng (lane 4).

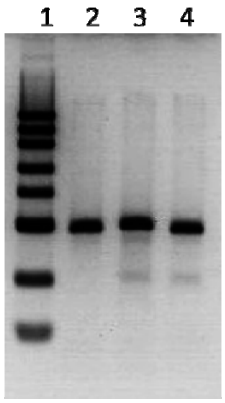
Cell Transfection and Gene Editing Efficiency Testing

The green fluorescence protein EGFP can be taken as a reporter to monitor the transfection efficiency and enrich the cell populations for desired genome edits. To test the transfection efficiency and gene editing efficiency of GenCrispr NLS-Cas9-EGFP, 20 μg NLS-Cas9-EGFP and 10 μg control gRNA were introduced into 293T cells by electrophoresis, which lead to over 90% transfection rate. Compared to NLS-Cas9 positive control, NLS-Cas9-EGFP caused a similar gene editing efficiency.

Left:



Right:



Left: Cell transfection assay. 12 h after electroporation, cells were observed under bright or fluorescence microscope. Right: in vivo gene editing efficiency assay by T7E1. Lane 1, marker; lane 2, negative control; lane 3, gRNA + NLS-Cas9-EGFP; lane 4, gRNA + NLS-Cas9.

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IX References

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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).
4. Kim, S., Kim, D., Cho, S.W., Kim, J., Kim, J.S, (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoprotein. Genome Res. 24(6), 1012-1019.

Note:

1. This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.
2. 1000 nM is equal to 190 ng/μl.

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