

SpCas9-NLS

Cat. No.: S1523005 | Pack size: 100pmol / 1000pmol | Storage: -20 °C

Overview

SpCas9-NLS is an RNA-guided endonuclease derived from *Streptococcus pyogenes*. Guided by crRNA and tracrRNA (or sgRNA formed by the two), this enzyme recognizes the position complementary to the sgRNA spacer sequence upstream of the PAM sequence (5'-NGG) in the double-stranded DNA target, and cleaves the DNA double strand just before the 3rd base upstream of the PAM. SpCas9-NLS is commonly used for gene editing. To improve intracellular editing efficiency, this product carries the nuclear localization signal (NLS) of the simian virus 40 (SV40) T antigen at the N-terminus. This product can also be used for in vitro target DNA cleavage and gene cloning, etc.

Components List

S1523005	Components	Appearance	100pmol	1000pmol	Storage
S1523005A	SpCas9-NLS (10 μM)	Liquid	10μl	100μl	-20°C. Avoid freeze/thaw cycle.
S1523005B	10× Cut Buffer C	Liquid	1ml	1ml	-20°C. Avoid freeze/thaw cycle.

Inactivation Condition

Incubate at 85°C for 5 min.

Quality Control

Protein Purity Determined by SDS-PAGE, the protein purity is ≥95%.

Non-specific Endonuclease Activity 10 pmol of SpCas9-NLS is incubated with 200 ng of supercoiled plasmid DNA at 37°C for 4 h. As determined by agarose gel electrophoresis, less than 10% of the plasmid DNA is converted to nicked or linear form.

DNase Activity 10 pmol of SpCas9-NLS is incubated with 15 ng of double-stranded DNA fragments at 37°C for 16 h. No change in the double-stranded DNA substrate is observed by agarose gel electrophoresis.

RNase Activity 10 pmol of SpCas9-NLS is incubated with 500 ng of total RNA at 37°C for 1 h. More than 90% of the RNA remains intact as determined by agarose gel electrophoresis.

Protocol In Vitro Cis Cleavage Assay

① Prepare the following reaction system on ice:

Reagent	Volume	Final Concentration
10× Cut Buffer C	2 µl	1×
SpCas9-NLS (10 µM) ^a	0.5 µl	250 nM
sgRNA (10 µM) ^{a,b}	0.5 µl	250 nM
Target DNA (1 µM) ^a	0.5 µl	25 nM
Nuclease-free water	To 20 µl	—

^a. If the cleavage products are detected by gel electrophoresis, it is recommended to use 100~500 ng of Target DNA with a length between 300 bp and 3 kb. It is recommended that the molar ratio of SpCas9-NLS : sgRNA : Target DNA is 10:10:1 to ensure complete cleavage of the target DNA as much as possible.

^b. sgRNA can be designed with reference to the following sequence:

5'-NNNNNNNNNNNNNNNNNNNNNGUUUAGAGCUAUGCUGAAAGCAUAGCAAGUAAAAUAAGGCUAG UCCGUUAUCAACUUGAAAAAGUGGCACCGAG-3' (The underlined part represents the spacer sequence complementary to the Target DNA specifically).

② Incubate at 37°C for 30 min~1 h, then inactivate at 85°C for 5 min.

③ Analyze the products by agarose gel electrophoresis.

Troubleshooting Problem

1: Incomplete cleavage of the target DNA is observed.

Possible causes & solutions:

Improper ratio of SpCas9 Nuclease, gRNA, and target DNA.

Solution: It is recommended that the molar ratio of SpCas9 Nuclease, gRNA, and target DNA is at least 10:10:1. The reaction can also be made more complete by appropriately extending the reaction time.

Related to the gRNA sequence.

Solution: Select a more suitable gRNA sequence according to the target DNA. The efficiency of different gRNAs can vary greatly.

gRNA degradation.

Solution: Verify the integrity of the gRNA by gel electrophoresis.

Inappropriate reaction buffer.

Solution: Please use the 10× Cut Buffer C provided with SpCas9 NLS.

Problem 2: Differences in digestion efficiency between different gRNAs.

Possible causes & solutions:

gRNA sequence design.

Solution: The designed gRNA needs to be verified against the template sequence.

gRNA quality.

Solution: Verify the integrity of the gRNA by agarose gel electrophoresis.

Specifications

Attribute	Value
Product Name	SpCas9-NLS
Synonyms	Cas9 NLS NLS-SpCas9 SpCas9 (NLS) Cas9-NLS SpyCas9-NLS NLS-Cas9 Nuclease
Grade	Animal Free, Recombinant, Suitable for molecular biology, EnzymoPure™
Specifications & Purity	Animal Free, Recombinant, Suitable for molecular biology, EnzymoPure™, ≥95%(SDS-PAGE), expressed in E.coli;10 μM
Accession #	Q99ZW2
Expression System	E. coli
Molecule Type	Enzyme

Storage and Shipping

Attribute	Value
Concentration	expressed in E.coli;10 μM
Storage	Store at -20°C,Avoid repeated freezing and thawing
Shipped In	Ice chest + Ice pads

Attribute	Value
Stability And Storage	Store at -20°C long term (24 months). Upon receipt, it is recommended to aliquot. Avoid freeze/thaw cycle.

Recommended Applications

SpCas9-NLS is commonly used for gene editing and can also be used for in vitro target DNA cleavage and gene cloning.

Safety & Precautions

1. Read the current SDS before use. Wear a lab coat, disposable gloves, and eye protection, and follow local laboratory safety and EHS procedures.
2. Store at -20 °C. Upon receipt, aliquot the product as appropriate and avoid repeated freezing and thawing.
3. Use nuclease-free consumables and reagents for the in vitro cleavage assay to minimize contamination and preserve assay performance.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

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- For Research Use Only (RUO). Products are supplied to verified businesses, institutions, and qualified professionals for research and development use only. Not for use in humans, animals, diagnosis, or therapy.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any product component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
- Performance depends on gRNA design, target DNA quality, reaction setup, handling, and operator technique. Users are responsible for validating the product for their specific application.
- Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document will be reviewed and reissued.