SpCas9-NLS

REF: EG24205-S/M

Storage Condition

-20℃

Components

Component	EG24205S	EG24205M
SpCas9-NLS (10 μM)	10 μl	100 μl
10× Cut Buffer C	1 ml	1 ml

Description

SpCas9-NLS is an RNA-guided endonuclease from *Streptococcus pyogenes*. Guided by crRNA and tracrRNA (or a sgRNA formed by their fusion), SpCas9-NLS recognizes the region immediately upstream of the PAM sequence (5'-NGG) in double-stranded DNA targets and produces a double-stranded break precisely three nucleotides upstream of the PAM.

SpCas9-NLS is commonly employed for gene editing. To enhance cellular editing efficiency, SpCas9-NLS contains a nuclear localization sequence (NLS) derived from Simian virus 40 (SV40) T antigen on N-termini. Additionally, this product can be utilized for the cleavage of target DNA in vitro and gene cloning, etc.

Heat Inactivation

Incubation at 85℃ for 5 minutes.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 1 μg of supercoiled plasmid and 10 pmol of SpCas9-NLS incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 10 pmol of SpCas9-NLS incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 pmol of SpCas9-NLS incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Protocol

In vitro cis cleavage experiment

① Prepare the following reaction mixture on ice:

Reagent	Amount	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 detect the products by agarose gel electrophoresis, it is recommended that the amount of target DNA is 100~500 ng, and the length of target DNA is between 300 bp and 3 kb. It is recommended that the molar ratio of SpCas9-NLS : sgRNA : Target DNA is 10:10:1, and try to ensure that the target DNA is completely cleaved.

b. sgRNA can be designed with reference to the following sequence:
5'-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAUGCUGAAAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUG-3' (The underline indicates the spacer sequence that is specifically complementary to Target DNA).

② Incubate at 37℃ for 30 minutes to 1 hour, and then incubate at 85℃ for 5 minutes for inactivation;

③ Detect the products by agarose gel electrophoresis.

FAQ & Troubleshooting

Problem	Possible Reason	Solution
Observe Incomplete digestion of the target DNA.	Incorrect ratio of Cas9 Nuclease to guide RNA, and target site.	For complete digestion we recommend a 10:10:1 or higher molar ratio of Cas9 Nuclease: guide RNA : target site. The reaction time can also be extended.
	It is related to the sequence of the gRNA.	Select a more suitable gRNA sequence based on the target DNA. The effectiveness of different gRNAs can vary significantly.
	Degradation of gRNA	Verify the integrity of the guide RNA by gel electrophoresis.
	The reaction buffer is not appropriate.	Please use the 10× Cut Buffer C that provided in this product.
There are differences in digestion efficiency between different gRNAs.	The sequence of gRNA	The designed gRNA needs to be validated for sequence and template.
	The quality of the gRNA	Verify the integrity of the gRNA by using agarose gel electrophoresis.

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