Tribioscience

saCas9 Nuclease NLS Protein

Catalog	Unit
TBP0209	32.5 µg

Description

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system is the latest RNA-guided endonuclease tool in genome editing which allows for very specific genomic disruption and replacement.

The Cas9 nuclease serves to unwind the genomic DNA duplex next to conserved protospacer adjacent motifs (PAMs) and homes in on its target sequence, which is recognized by a complementary single-guide RNA. The resulting double-stranded break gets repaired by the non-homologous end joining (NHEJ) pathway, leading to a disruption in the open reading frame of the targeted gene. Alternatively, by supplying a suitable repair template, virtually any desired point mutation can be introduced at the break point via homology-directed repair (HDR).

The Cas9 nuclease from the bacteria *Staphylococcus aureus*, abbreviated saCas9, is gaining popularity as an alternative to spCas9 due to its relatively smaller size. The saCas9 PAM sequence is 5'-NNGRRN (preferably 5'-NNGRRT). saCas9 Nuclease NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Component

Product Component	Quantity
saCas9 Nuclease NLS Protein	25 µl (250 pmol, 10 µM)
10X Cas9 Reaction Buffer	1.25 ml

Store at -20°C.

Protocol

In vitro digestion of DNA

1. Add the following components to a sterile, nuclease-free tube sitting on ice:

Product Component	Volume
sgRNA (300 nM)	3 µl
saCas9 Nuclease NLS Protein (1 µM)[1]	1µl
10X Cas9 Reaction Buffer	3 µl
Nuclease-free H_2O	20 µl
Pre-incubate for 15 minutes at 37°C	
Substrate DNA (30 nM)	3 µl

[1]Dilute to 1 µM. See General Notes for further details.

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 30 minutes.
3. Analyze fragments via agarose gel electrophoresis.

General Notes

- Dilute saCas9 Nuclease NLS Protein (10 µM) to 1 µM using the following:
  - 10X Cas9 Reaction Buffer for immediate use.
  - 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol if storing in -20°C before use.
- The substrate DNA: sgRNA: saCas9 molar ratio must be kept at 1:10:10 for highest efficiency.

For research use only