

saCas9 Null Mutant NLS Protein

Catalog
TBP0201

Unit
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 saCas9 Null Mutant NLS Protein is created by mutating both cleavage domains of the wild type saCas9. Such a saCas9 protein retains its ability to bind to genomic DNA through gRNA:genomic DNA base pairing, however, the saCas9 Null Mutant does not introduce any genome modifications. Therefore, this protein can provide a useful negative control for CRISPR experiments. In addition, binding of the Null Mutant can act as a roadblock to hinder transcription, thus offering a useful tool to achieve reversible knock-down of gene expression.

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 NLS Null Mutant contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Component

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

Store at -20°C.

Protocol

Reaction Conditions

Use 1X Cas9 Reaction Buffer and incubate at 37 °C.

General Notes

- Dilute saCas9 Null Mutant 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.
- saCas9 Null Mutant NLS Protein is suitable for use in imaging of genomic loci in living cells and fixed cells as well as for gene expression regulation.

For research use only