saCas9 Null Mutant Protein

Cat. No. K146
Store at -20°C.

Product 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 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 sgRNA:genomic DNA base pairing, however, the saCas9 Null Mutant does not induce cleavage. 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).

Product Component	Quantity	Part No.
saCas9 Null Mutant Protein	25 µl (250 pmol, 10 µM)	K146
10X Cas9 Reaction Buffer	1.25 ml	K000

Protocol

Reaction Conditions

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

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