Cas9 Null Mutant NLS Protein

Cat. No. K142

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 Cas9 Null Mutant NLS Protein is created by mutating both cleavage domains of the wild type Cas9 (D10A and H840A). Such a Cas9 protein retains its ability to bind to genomic DNA through gRNA:genomic DNA base pairing, however, unlike Cas9 Nuclease and Cas9 Nickase, where permanent gene disruption can be achieved, the Cas9 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 *Streptococcus pyogenes*, abbreviated spCas9, is the most commonly used Cas9 variant. The reason for spCas9 popularity is two-fold. First the spCas9 PAM sequence is 5'-NGG, which is highly abundant in the genome allowing virtually any gene to be targeted. The spCas9 enzyme also has on average a higher efficiency *in vivo* compared to other variants. Cas9 Null Mutant NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Product Component	Quantity	Part No.
Cas9 Null Mutant NLS Protein	25 µl (250 pmol, 10 µM)	K142
10X Cas9 Reaction Buffer	1.25 ml	K000

Protocol

Reaction Conditions

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

General Notes

- Dilute Cas9 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 : Cas9 molar ratio must be kept at 1:10:10 for highest efficiency.
- Cas9 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.