

Cas9 Null Mutant GFP NLS Protein

Catalog
TBP0190

Unit
9.5 μ g

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 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 from the bacteria *Streptococcus pyogenes*, abbreviated spCas9, is the most commonly used Cas9 variant. The fusion of Cas9 to GFP allows for visual confirmation of transfection as well as subsequent verification of Cas9 clearance from the cells. Cas9 Null Mutant GFP can also be used for FACS applications and screening. Cas9 D10A Null Mutant GFP NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Component

Product Component	Quantity
Cas9 Null Mutant GFP NLS Protein	50 μ l (50 pmol, 1 μ M)
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 Cas9 Null Mutant GFP NLS Protein (10 μ M) to 1 μ M 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 GFP 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