

Cas9 Nuclease GFP NLS Protein

Catalog
TBP0189

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 fusion of Cas9 Nuclease NLS to GFP allows for visual confirmation of transfection as well as subsequent verification of Cas9 clearance from the cells. Cas9 Nuclease-GFP can also be used for FACS applications and screening. Cas9 Nuclease-GFP NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Component

Product Component	Quantity
Cas9 Nuclease GFP NLS Protein	50 μ l (50 pmol, 1 μ M)
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:

Component	Volume
sgRNA (300 nM)	3 μ l
Cas9 Nuclease GFP NLS Protein (1 μ M) ¹	1 μ l
10X Cas9 Reaction Buffer	3 μ l
Nuclease-free H ₂ O	20 μ l
Pre-incubate for 15 minutes at 37°C	
Substrate DNA (30 nM)	3 μ l

¹Dilute to 1 μ M. See General Notes for further details.

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 1 hour.
3. Analyze fragments via agarose gel electrophoresis.

General Notes

- Dilute Cas9 Nuclease 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.

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