



Cas9 Nuclease GFP NLS Protein

Cat. No. K048, K148

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 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.

Product Component	Quantity	Part No.
Cas9 Nuclease GFP NLS Protein	50 µl (50 pmol, 1 µM)	K048
10X Cas9 Reaction Buffer	1.25 ml	K000
Cas9 Nuclease GFP NLS Protein	25 µl (250 pmol, 10 µM)	K148
10X Cas9 Reaction Buffer	1.25 ml	K000

Protocol

In vitro digestion of DNA

1. Add the following components to a sterile, nuclease-free tube sitting on ice:

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

¹ Dilute to 1 µM. 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 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.