

Gene Snipper™ Cas9 Nickase GFP NLS

ALTERNATIVE NAMES: Cas9-GFP (D10A); CRISPR-associated endonuclease
Cas9(D10A) from *Streptococcus pyrogenes*

CATALOG #:
M1285-50 50 pmol (50 µl)
M1285-250 250 pmol (25 µl)

SOURCE: Recombinant *E. coli* and contains His-tag

CONCENTRATION:
M1285-50 1000 nM
M1285-250 10 µM

FORM: Enzyme supplied with 10X Reaction Buffer

COMPONENTS:

Product Name	M1285-50	M1285-250	Part No.
Cas9 Nickase GFP NLS	1000 nM	10 µM	M1285-XX-1
10X Cas9 Reaction Buffer	1.25 ml	1.25 ml	M1285-XX-2

ENZYME STORAGE BUFFER: 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol

10X CAS9 REACTION BUFFER: 200 mM HEPES, 50 mM MgCl₂, 1 M NaCl, 1 mM EDTA, pH 6.5

STORAGE CONDITIONS: Store all components at -20°C. Avoid repeated freeze/thaw cycles. All components are stable for 1 year from the date of shipping when stored and handled properly.

DESCRIPTION: The Cas9 nuclease has been modified into a “nickase” so that one of its catalytic domains is inactive, resulting in a single stranded nick instead of a double stranded break at the target site on the genomic DNA. In this system not one, but two gRNAs located in close proximity (<20 nucleotides) on opposite strands of the genomic DNA are required in order to create the desired double stranded break. An undesired single stranded nick in the genomic DNA can be quickly repaired by the Homology Directed Repair (HDR) pathway using the other intact strand as the template.

The fusion of Cas9 (D10A) Nickase NLS to GFP allows for visual confirmation of transfection as well as subsequent verification of Cas9 clearance from the cells. Cas9 D10A Nickase GFP can also be used for FACS applications and screening. Cas9 D10A Nickase GFP NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

PROTOCOL: *In vitro* digestion of DNA

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

Components	Volume	Final Concentration
sgRNA #1 (300 nM)	3 µl	~30 nM
sgRNA #2 (300 nM)	3 µl	~30 nM
Cas9 (D10A) Nickase GFP NLS Protein (1000 nM)	1 µl	~30 nM
10X Cas9 Reaction Buffer	3 µl	1X
Nuclease-free H ₂ O	20 µl -	-
Pre-Incubate for 15 minutes at 37°C		
Substrate DNA (30 nM)	3 µl	3 nM
Total Volume	30 µl	-

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 1 hour
3. Analyze fragments via agarose gel electrophoresis. *Note: The substrate DNA: sgRNA: Cas9 molar ratio must be kept at 1:10:10 for highest efficiency.*

RELATED PRODUCTS:

- Gene Snipper™ Cas9 Protein (Cat. No. M1094-50, -250)
- Gene Snipper™ Cas9 NLS (Cat. No. M1095-50, -250)
- Gene Snipper™ Cas9 Nickase (D10A) (Cat. No. M1096-50, -250)
- Gene Snipper™ Cas9 (D10A) NLS (Cat. No. M1097-50, -250)
- Gene Snipper™ Cas9 Nickase (H840A) (Cat. No. M1098-50, -250)
- Gene Snipper™ Cas9 (H840A) NLS (Cat. No. M1099-50, -250)
- Gene Snipper™ Cas9 Null (Cat. No. M1100-50, -250)
- Gene Snipper™ Cas9 Null NLS (Cat. No. M1103-50, -250)
- Gene Snipper™ CRISPR Activity Kit (Cat. No. K1104-25)
- Gene Snipper™ SaCas9 Protein (Cat. No. M1280-50, -250)
- Gene Snipper™ SaCas9 NLS (Cat. No. M1281-50, -250)
- Gene Snipper™ SaCas9 Null (Cat. No. M1282-50, -250)
- Gene Snipper™ SaCas9 Null NLS (Cat. No. M1280-50, -250)
- Gene Snipper™ Cas9 GFPNLS (Cat. No. M1284-50, -250)
- Gene Snipper™ Cas9 Nickase GFPNLS (Cat. No. M1285-50, -250)
- Gene Snipper™ Cas9 GFPNull NLS (Cat. No. M1286-50, -250)

FOR RESEARCH USE ONLY! Not to be used on humans.