BioVision

Rev 01/19

For research use only

Gene Snipper™ Cas9 Nickase (D10A)

CATALOG#:

M1096-50	50 pmol (50 µl, 8.0 µg)
M1096-250	250 pmol (25 µl, 40 µg)

SOURCE: Recombinant *Streptococcus pyogenes* Cas9 (CRISPR associated protein 9) Nickase (D10A) protein purified from *E. coli*

MOLECULAR WEIGHT: ~160 kDa

PURITY: The protein is at least 95% pure by SDS PAGE

CONCENTRATION:

M1096-50	1000 nM (160 µg/ml)
M1096-250	10 µM (1.60 mg/ml)

FORM: Colorless liquid.

KIT COMPONENTS:

Product Components	Concentration	Part No.
Cas9 Nickase D10A Protein	50 pmol (50 µl)	M1096-50-1
10X Cas9 Reaction Buffer	1.25 ml	M1096-50-2
Product Components	**Concentration**	**Part No.**
Cas9 Nickase D10A Protein	250 pmol (25 µl)	M1096-250-1
10X Cas9 Reaction Buffer	1.25 ml	M1096-250-2

10X CAS9 REACTION BUFFER COMPONENTS: 200 mM HEPES, 50 mM $MgCl_2$, 1 M NaCl,1 mM EDTA, pH 6.5).

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.

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.

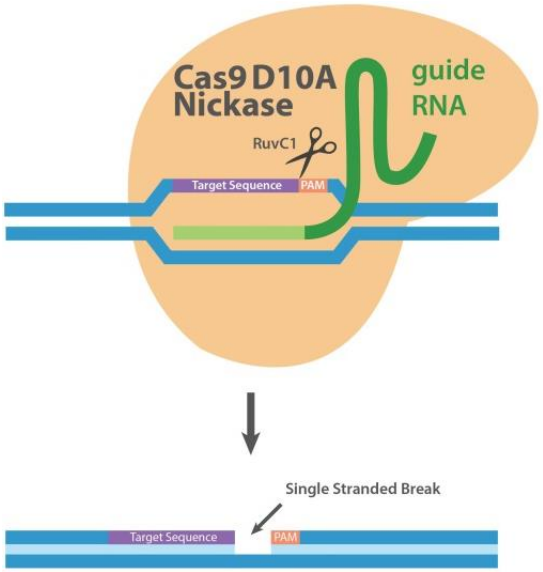
REACTION CONDITIONS: Use 1X Cas9 Reaction Buffer and incubate at 37°C

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. One concern with the current CRISPR Cas9 technology is the potential off-target effects of the Cas9 nuclease.

To counteract off-target mutagenic effects of this system, the Cas9 Nickase D10A was developed with a D10A mutation in its RuvC1 nuclease domain. Unlike the Cas9 nuclease, this mutant form generates a single-stranded nick instead of a double-strand break (DSB). Because a single DNA nick is quickly repaired with high fidelity by the cellular machinery, the system requires two closely juxtaposed nicks in order to trigger the same genomic disruption as the Cas9 nuclease. This effectively boosts the recognition sequence to 40 instead of 20 nucleotides, and, as a result, off-target effects become highly unlikely. Thus, the double-nickase CRISPR system offers unparalleled specificity to satisfy even the most stringent of experimental requirements.

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.

BIOLOGICAL ACTIVITY: The activity of the protein in *in vivo* is confirmed by CRISPR Genome Cleavage Detection Kit.



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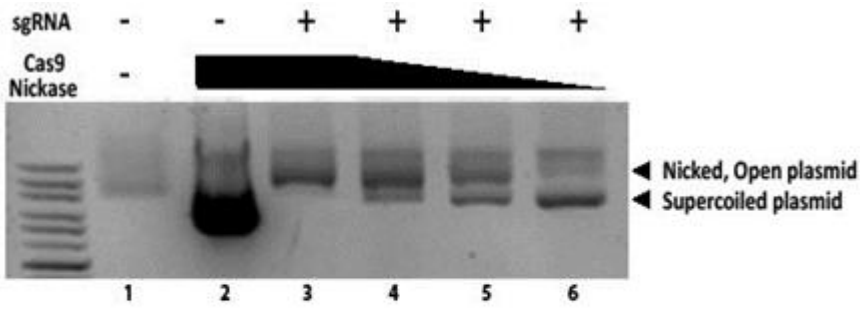

Legend
Lane 1: Freeze thawed (nicked) supercoiled plasmid
Lane 2: 100 nM Cas9 Nickase D10A Protein (no sgRNA)
Lane 3: 100 nM Cas9 Nickase D10A Protein + sgRNA
Lane 4: 50 nM Cas9 Nickase D10A Protein + sgRNA
Lane 5: 25 nM Cas9 Nickase D10A Protein + sgRNA
Lane 6: 10 nM Cas9 Nickase D10A Protein + sgRNA

Ability of Cas9 Nickase D10A protein to nick supercoiled plasmid to become an open circle.

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)

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