BioVision

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For research use only

Gene Snipper™ Cas9 Protein

CATALOG#: M1094-50 50 pmol (50 µl)
M1094-250 250 pmol (25 µl)

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

MOLECULAR WEIGHT: ~160 kDa

PURITY: The protein is at least 95% pure by SDS PAGE. It does contain a His-tag.

CONCENTRATION: M1094-50 1000 nM
M1094-250 10 µM

FORM: Colorless liquid.

KIT COMPONENTS:

Product Components	Concentration	Part No.
Cas9 Protein	50 pmol (50 µl)	M1094-XX-1
10X Cas9 Reaction Buffer	50 pmol (1.25 ml)	M1094-XX-2

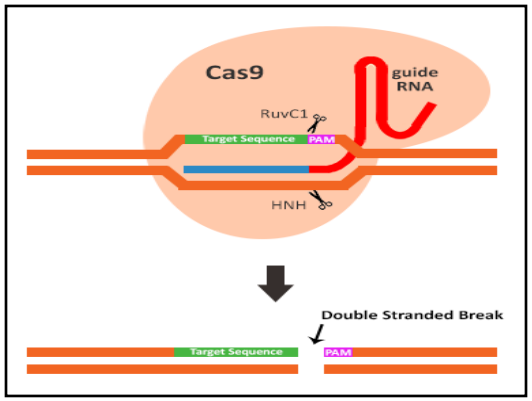
10X CAS9 REACTION BUFFER COMPONENTS: 200 mM HEPES, 50 mM $MgCl_2$, 1 M NaCl,1 mM EDTA, pH 6.5)

RECONSTITUTION: 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.

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. Cas9 (CRISPR associated protein 9) is an RNA-guided DNA endonuclease enzyme associated with the CRISPR adaptive immunity system in *Streptococcus pyogenes*, among other bacteria. *S. pyogenes* utilizes Cas9 to memorize and later interrogate and cleave foreign DNA such as invading bacteriophage DNA or plasmid DNA. Cas9 Nuclease protein forms a very stable ribonucleoprotein (RNP) complex with the guide RNA (sg RNA) of the CRISPR/Cas9 system and unwinds the genomic DNA duplex and cleaves both strands upon recognition of the target sequence by the sg RNA. The resulting double-stranded break gets repaired by the non-homologous end joining (NHEJ) pathway, leading to a disruption in the open reading frame of the targeted gene.

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



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 (300 nM)	3 µl	~30 nM
Cas9 Nuclease Protein (1000 nM)	1 µl	~30 nM
10X Cas9 Reaction Buffer	3 µl	1X
Nuclease-free H_2O	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)

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

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