

SPECIFICATION SHEET

Product Name: SpCas9
Catalog # CS09-E311H

Product Description: Recombinant full-length *S. pyogenes* SpCas9 expressed in *E. coli* with a C-terminal His tag. Enzyme sold with 1 mL of Cas9 reaction buffer, 10x (CS09-09).

Storage and Stability: Store at -70°C . To avoid repeated handling and multiple freeze/thaw cycles, aliquot product into smaller quantities.

Data for a Sample Lot:

Alternate Names

: Cas9, Csn1, Spy_1046

Source

 : *Streptococcus pyogenes*
Accession Numbers

 : Genbank: [AAK33936.1](https://www.ncbi.nlm.nih.gov/nuclot/AAK33936.1)

 UniProt: [Q99ZW2](https://www.uniprot.org/uniprot/Q99ZW2)
Host

 : *E. coli*
Formulation

: Protein stored in 50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 150 mM imidazole, 1 mM DTT, 10% glycerol

Molecular Weight

: Calculated: 159.7 kDa

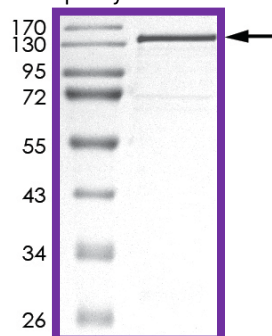
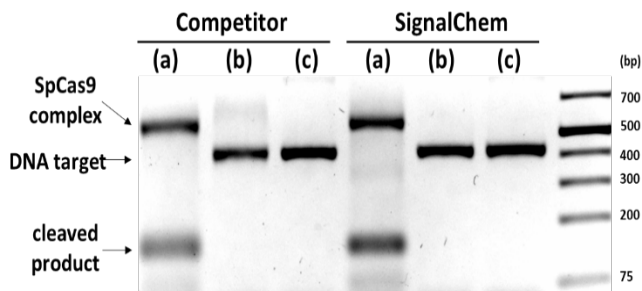
Observed: ~150 kDa

Concentration

 : 0.5 $\mu\text{g}/\mu\text{L}$

 6.26 pmol/ μg
Purity

: The purity was determined to be >80% by densitometry

Gel Image

Activity


SpCas9-mediated specific endogenous DNA cleavage. The reactions consist of (a) target DNA, crRNA, tracrRNA, and SpCas9; (b) target DNA, crRNA, and SpCas9; (c) target DNA and crRNA.

Recommendation

: Always include RNase inhibitor at recommended concentrations in Cas enzyme reactions.

Scientific Background

: *Streptococcus pyogenes* Cas9 is a type II-A protein used in most current CRISPR-based genetic engineering methodologies (1). CRISPR RNAs (crRNAs) pairs with trans-activating RNAs (tracrRNA) to direct sequence-specific cleavage by Cas9, introducing double-stranded breaks in cell-invading DNA in bacterial and archaeal CRISPR/Cas9 systems (1,2). Cas9 requires the 5'-NGG-3' protospacer adjacent motif (PAM) to destabilize the target DNA (3). Cas9's HNH endonuclease domain cleaves the strand complementary to the crRNA while its RuvC endonuclease domain cleaves the non-complementary strand (2).

References

- : 1. Jinek, M. et al. 2014. Structures of Cas9 Endonucleases Reveal RNA-Mediated Conformational Activation. *Science*. 343(6176): 1247-1251.
2. Jinek, M. et al. 2012. A programmable dual RNA-guided endonuclease in adaptive bacterial immunity. *Science*. 337(6096): 816-821.
3. Anders, C. et al. 2014. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature*. 513(7519): 569-573.