



CRISPR/Cas9 nuclease

In vivo/in vitro gene editing

Contents:

CRISPR/Cas9 is provided at a concentration of 10 pmol/ μ L (10 μ M).

Background

CRISPR/Cas9 is a *Streptococcus pyogenes* Type II Cas9 endonuclease that can be programmed by a small RNA to site-specifically digest DNA. The specificity of cleavage is determined by the sequence of a single guide RNA (sgRNA) that contains bases that are complimentary to the target site. The simplicity of the CRISPR/Cas9 endonuclease system enables precise *in vitro* DNA digestion.

Application Notes

CRISPR/Cas9 can be programmed to cleave DNA precisely by the sequence of the sgRNA that is loaded onto the protein to cleave DNA virtually anywhere. This enables site-specific *in vitro* engineering of genomic, plasmid, or PCR amplified DNA.

**These products are intended for research use only, not for diagnostic use. The safety and efficacy of these products in diagnostic or other clinical uses has not been established.*

Protein Details

CRISPR/Cas9 is expressed in *E. coli* fused to a solubility-enhancing protein. The molecular weight of this fusion protein is 171 kDaltons.

Shipping & Storage

CRISPR/Cas9 is stored at -20 °C in 50% glycerol, 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, pH 7.5. *Can be supplied in a glycerol-free buffer as a custom order.*

CRISPR/Cas9 is shipped on dry or blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided

Quality Control

- CRISPR/Cas9 activity: >90% cleavage of 1 nM, 4 kb PCR product in 60 minutes at 37 °C using 40 nM targeted gRNA and 1 pmol CRISPR/Cas9 in 50 μ L (20 nM).
- Purity: >95% as determined by SDS-PAGE analysis
- <0.2 ng contaminating host DNA per pmol

In vitro digestion of DNA with CRISPR/Cas9 nuclease

Product Overview

CRISPR/Cas9 is a recombinant form of *Streptococcus pyogenes* Type II Cas9 nuclease that site specifically digests DNA using the complementarity sequence of a small RNA bound to the protein to guide the nuclease to its target.

A protocol is provided for *in vitro* digestion of double stranded DNA with CRISPR/Cas9 and a single guide RNA.

Useful Information

The DNA, guide RNA and nuclease-free water for this protocol are not supplied with the CRISPR/Cas9.

Information for the design of a Cas9 nuclease guide RNA containing a target sequence can be viewed at the Addgene website.

10X Reaction Buffer is used in the protocol below. 10X Reaction Buffer is 500 mM NaCl, 100 mM Tris-HCl, 100 mM MgCl₂, 10 mM DTT, pH 7.9. This buffer can be substituted with 10X TA Buffer. 10X TA Buffer is 330 mM Tris acetate, 660 mM KCl, and 100 mM magnesium acetate, pH 7.5.

General Protocol

- 1) Assemble the reaction components at room temperature in a microfuge tube.
- 2) Mix the reaction components and incubate at 37 °C for 1 hour.
- 3) Stop the reaction by heating at 70 °C for 15 min.

Component	Volume
Nuclease-free water	20 µL
10X Reaction Buffer	3 µL
300 nM guide RNA	3 µL (30 nM final)
1 µM CRISPR/Cas9 NLS	1 µL (~30 nM final)
Pre-incubate the above mixture at RT for 10 min before adding DNA	
30 nM DNA	3 µL (30 nM final)
Total reaction volume	30 µL