Intact Genomics®

Cas9 Nuclease

Catalog #	3272	3273 (HC)	3276 (HC)
Package Size	40 µg (250 pmol)	80 µg (500 pmol)	400 µg (2,500 pmol)
Volume	250 µl	50 µl	250 µl
Concentration	160 ng/µl (1 µM)	1,600 ng/µl (10 µM)	

CRISPR-associated (Cas) systems

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids (1). The CRISPR system consists of a short non-coding guide RNA (sgRNA) made up of a target complementary CRISPR RNA (crRNA) and an auxiliary transactivating crRNA (tracrRNA). The sgRNA guides the Cas9 endonuclease to a specific genomic locus via base pairing between the crRNA sequence and the target sequence, and cleaves the DNA to create a double-strand break. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif) (1). The PAM sequence, NGG, must follow the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence (Fig.1).

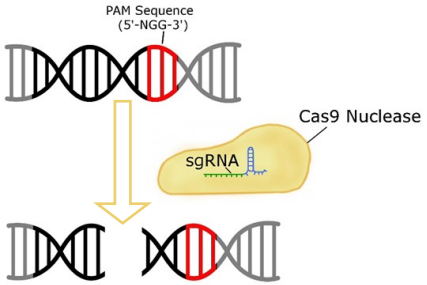

Fig. 1: Overview of the CRISPR-associated (Cas) systems.

Description

Intact Genomics Cas9 Nuclease is the purified recombinant Streptococcus pyogenes Cas9 enzyme containing a nuclear localization signal (NLS) at the C-terminal for targeting to the nucleus. This enzyme is designed to perform CRISPR/Cas9-mediated genome editing (1, 2). The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (Fig. 2).

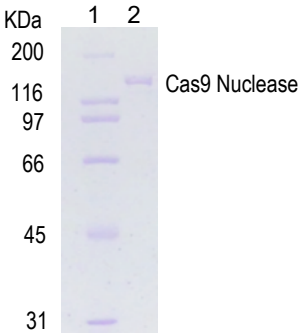

Fig. 2: Lane 1. Protein Marker
Lane 2. Cas9 Nuclease

Product Source

E. coli BL21 (DE3) strain expressing a Cas9 gene from Streptococcus pyogenes with an N-terminal 6xHis tag and C-terminal SV40 nuclear localization signal (NLS).

Product Includes

1) Cas9 Nuclease
2) 10x Cas9 Nuclease Reaction Buffer

Storage Temperature

–20 °C

Storage Buffer

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC

1x Cas9 Reaction Buffer

20 mM HEPES
100 mM NaCl
5 mM $MgCl_2$
0.1 mM EDTA
pH 6.5 @ 25 °C

Quality Control Assays

Cas9 nuclease is free from detectable RNase, Endonuclease (nicking) and non-specific DNase activities.

Functional Testing

Cas9 Nuclease functional testing was done by in vitro DNA cleavage assay with the following protocol which gives more than 95% digestion of the substrate DNA as determined by agarose gel electrophoresis (Fig. 3).

1) Set up 30 µl reaction in a microcentrifuge tube on ice with the following combinations.

Target DNA	x µl (~100 ng)
sgRNA	x µl (~4000 ng)
10x Cas9 Reaction Buffer	3.0 µl
Cas9 Nuclease	1.0 µl (~160 ng)
Add H_2O up to	30.0 µl

2) Gently mix the reaction mixture and centrifuge briefly.
3) Incubate at 37 ºC for 60 min.
4) Add 1 µl RNase (4 mg/ml)
5) Incubate at 37 ºC for 20 min.
6) Run 0.7 to1% agarose TBE gel.

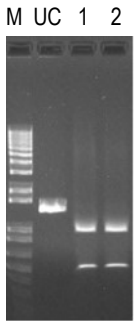

Fig. 3: M: Marker, UC: Uncut, 1: 1 µl Cas9, 2: 2 µl Cas9

Intact Genomics, Inc. 1100 Corporate Square Drive, Suite 257, St. Louis, MO 63132-2933, USA **Phone** 314.942.3655 **Fax:** 314.942.3656 **Email** sales@intactgenomics.com **www.intactgenomics.com**

Reference

1. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. Aug 17;337(6096):816-21.
2. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. (2013) RNA-guided human genome engineering via Cas9. Science. Feb 15;339 (6121):823-6.

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