Cas9 Nuclease

Catalog # EN301

Version 5.1

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Introduction

Cas9 Nuclease is a double-strand DNA endonuclease that uses a single guide RNA (sgRNA) to specify the site of cleavage. sgRNA contains complementary region for specific DNA binding, and can lead the Cas9 Nuclease to the target DNA. Cas9 Nuclease contains two nuclease domains, which can cut each strand of DNA duplex to produce double strand break. The cutting site locates at 3 bp from NGGPAM of the complementary region of target DNA.

Package Information

Components	EN301-01 (50 pmol)	EN301-02 (250 pmol)
Cas9 Nuclease	50 μl	250 μl
Cas9 Nuclease Reaction Buffer (10×)	1 ml	1 ml

Storage

All components should be stored at -20°C

Reaction Condition

Incubate at 37°C with 1× Cas9 Nuclease Reaction Buffer.

Source

Purified from a strain of recombinant E. coli that carries the Cas 9 gene of Streptococcus pyogenes.

Unit Definition

One unit (U) is defined as the amount of enzyme required to add 0.5 pmol of dNTP into acid insoluble sediments by reaction at 30°C for 10 min.

Quality Control

Protein purity: Purity of Cas9 Nuclease is high than 95% determined by SDS-PAGE with coomassie blue staining.

RNase activity: 40 ng of RNA and 1 pmol of Cas9 Nuclease were added into 10 μl Cas9 Nuclease reaction solution, and incubated at 37°C for 4 hours. Integrity of RNA is higher than 90% determined by agarose gel electrophoresis.

Endonuclease activity: 0.6 μg of supercoiled plasmid pBR322 and 1 pmol of Cas9 Nuclease were added into 50 μl Cas9 Nuclease reaction solution, and incubated at 37°C for 4 hours. No degradation was detected by agarose gel electrophoresis.

Exonuclease activity: 1 μg of λHind III and 1 pmol of Cas9 Nuclease were added into 50 μl Cas9 Nuclease reaction solution, and incubated at 37°C for 4 hours. No degradation was detected by agarose gel electrophoresis.

Application

Genome editing
in vitro digestion of DNA

Protocol for *in vitro* DNA digestion with Cas9 Nuclease

To obtain maximum cleavage efficiency, it is highly recommend to use the molar ratio of Cas9 Nuclease, sgRNA, and target DNA at 10:10:1 or higher. Total volume of reaction is 30 μl, which can be scaled up as needed. Dilute sgRNA with nuclease free water to 300 nM and DNA to 30 nM in advance.

1. Prepare the following reaction solution in order as follows:

Nuclease-free water	20 μl
10× Cas9 Nuclease Reaction Buffer	3 μl
sgRNA (300 nM)	3 μl
Cas9 Nuclease (1 μM)	1 μl
Total Volume	27 μl

2. Incubate at 37°C for 10 min.

3. Add 3 μl of 30 nM DNA.

4. Vortex to mix well and spin down briefly to collect the liquid.

5. Incubate at 37°C for 1 hour.

6. The digested product can be analyzed by agarose electrophoresis.

Note: Please wear gloves and hygiene mask, use nuclease-free consumables to avoid contamination of RNA nuclease during the operation.

Reference Result

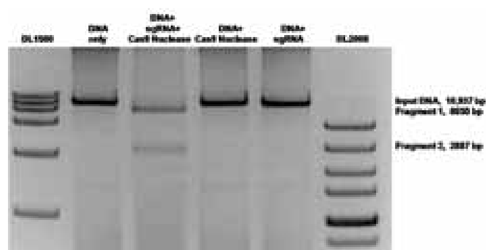

Fig. 1. Analysis of 11 kb dsDNA cutting with Cas9 Nuclease by agarose electrophoresis.