fnCpf1 Nuclease NLS Protein

Store at -20°C

Cat. No.	Description	Concentration	Quantity
K087	fnCpf1 Nuclease NLS Protein	10 µM	250 pmol, 25 µl
K187	fnCpf1 Nuclease NLS Protein	10 µM	2.5 nmol, 250 µl

Product Description

Using Cpf1 (a.k.a. Cas12a) in your CRISPR experiment offers several advantages over other CRISPR-associated nucleases.

- Due to the T-rich PAM sequence (TTTN), Cpf1 enables editing in regions unable to be targeted by Cas9.
- Cpf1 can be used with a shorter guide RNA (called crRNA) than Cas9.
- Cpf1 creates a staggered cut in dsDNA instead of a blunt cut.
- Cpf1 cuts distal to the PAM sequence, which may allow for multiple rounds of cleavage.

fnCpf1 is from the bacteria *Francisella novicida*. This protein contains a SV40 T antigen nuclear localization signal (NLS) on the N-terminus of the protein. If the cut caused by fnCpf1 is repaired by non-homologous end joining (NHEJ), an indel may be formed that disrupts the open reading frame of the targeted gene, leading to gene knockout. Alternatively, by supplying a repair template, a sequence can be knocked in at the cleavage site via homology directed repair (HDR).

Kit Components

Component	K087	K187
fnCpf1 Nuclease NLS Protein (10 µM)	25 µl	250 µl
10X Cpf1 Reaction Buffer (K100)	1.25 ml	1.25 ml

Product Source: Recombinant *E. coli*

Storage Conditions

Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for 1 year from the date of shipping when stored and handled properly.

Enzyme Storage Buffer

10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol.

10X Cpf1 Reaction Buffer Components

200 mM HEPES (pH 7.5), 1.5 M KCl, 100 mM MgCl2, 5 mM DTT

Protocol

***In vitro* digestion of DNA**

1. Add the following components to a sterile, nuclease-free tube on ice:

Components	Volume	Final Concentration
crRNA (300 nM)	3 µl	~30 nM
fnCpf1 Nuclease NLS Protein (1 µM)	1 µl	~30 nM
10X Cpf1 Reaction Buffer	3 µl	1X
Nuclease-free H_2O	20 µl	-
Pre-incubate for 30 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 30 minutes to 1 hour.
3. Analyze fragments via agarose gel electrophoresis

Note:
The substrate DNA : crRNA : Cpf1 molar ratio should be kept at 1:10:10 for highest efficiency.

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