Cas12a (asCpf1) Nuclease NLS Protein

Cat. No. K188

Store at -20°C.

Product Description

Using Cas12a (asCpf1) 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.

asCpf1 is from the bacteria *Acidaminococcus*. This protein contains a SV40 T antigen nuclear localization signal (NLS) on the N-terminus of the protein. If the cut caused by asCpf1 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).

Product Component	Quantity	Part No.
Cas12a (asCpf1) Nuclease NLS Protein	250 µl (2.5 nmol, 10 µM)	K188
10X Cpf1 Reaction Buffer	1.25 ml	K100

Protocol

In vitro digestion of DNA

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

Product Component	Volume
crRNA (300 nM)	3 µl
Cas12a (asCpf1) Nuclease NLS Protein (1 µM)[1]	1µl
10X Cpf1 Reaction Buffer	3 µl
Nuclease-free H_2O	20 µl
Pre-incubate for 30 minutes at 37°C	
Substrate DNA (30 nM)	3 µl

[1] Dilute to 1 µM. See General Notes for further details.

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.

General Notes

- Dilute Cas12a (asCpf1) Nuclease Protein (10 µM) to 1 µM using the following:
  - **10X Cpf1 Reaction Buffer** for immediate use.
  - **10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol** if storing in -20°C before use.
- The substrate DNA : crRNA : Cpf1 molar ratio must be kept at 1:10:10 for highest efficiency.

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