Cas12a Nuclease

Manual

Catalog #	3370	3373	3376
Package Size	40 µg	80 µg	400 µg

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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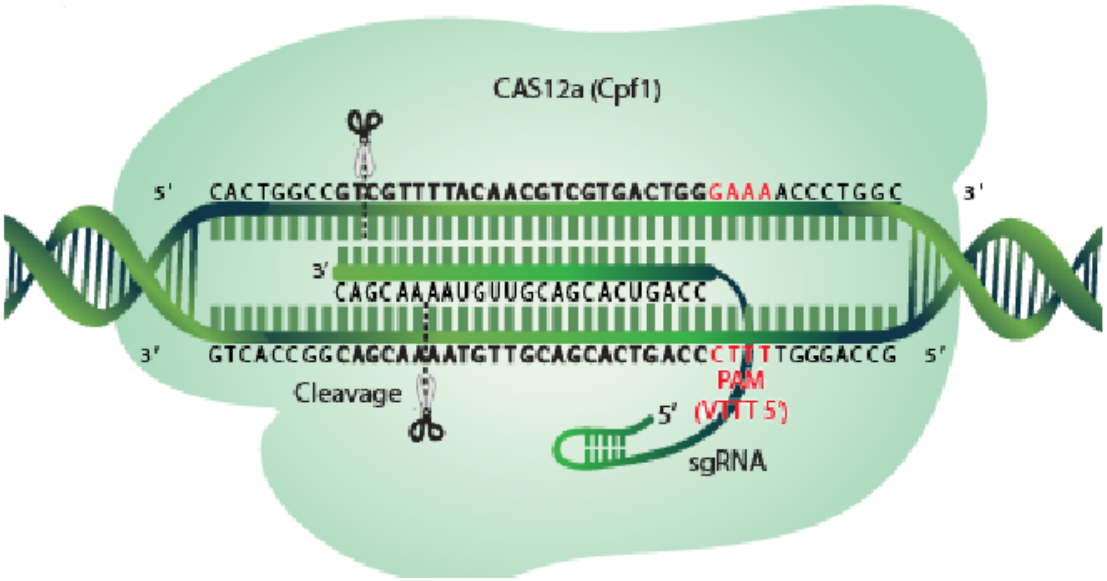
Description:

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 Cas12a (Cpf1) endonuclease to a specific genomic locus via base pairing between the crRNA sequence and the target sequence, and cleaves the DNA to create a "sticky" double-strand break with a four base pair overhang, which is different from the Cas9 generated blunt end break. The location of the break is within the target sequence 18 bases from the TTTV (V is A, C or G) PAM (Protospacer Adjacent Motif) (2). The PAM sequence must precede the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence (Fig.1).

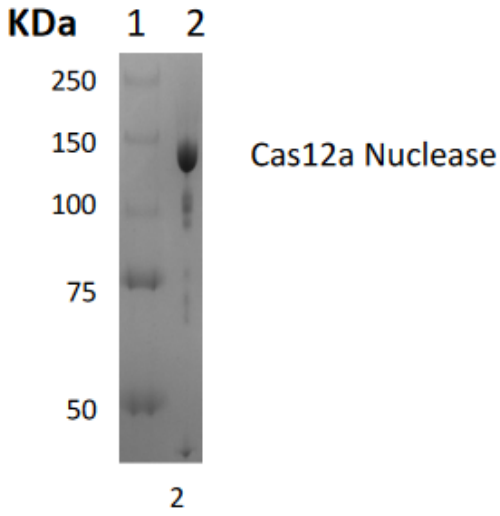
Fig. 1: Overview of the CRISPR-associated (Cas) systems.



Description:

Intact Genomics (ig®) Cas12a Nuclease is the purified recombinant Francisella tularensis Cas12a enzyme for *in vitro* editing. This enzyme is designed to perform CRISPR/Cas12a-mediated genome editing (1-3). The physical purity of this enzyme is ≥65% as assessed by SDS-PAGE with Coomassie® blue staining and Densitometry.

Fig. 2: Lane 1. Protein Marker
Lane 2. Cas12a Nuclease



Product Source:

E. coli BL21 (DE3) strain expressing a Cas12a (Cpf1) gene from Francisella tularensis with an N-terminal 6xHis tag.

Components and Storage:

Cas12a Nuclease Kits contain the below items. **Store all components at -20°C**.

- Cas12a Nuclease
- 10x Cas12a Nuclease Reaction Buffer
- Storage Buffer

  50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC
- 1x Cas12a Reaction Buffer

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

Quality Control:

Cas12a nuclease is free from detectable RNase, Endonuclease (nicking) and non-specific DNase activities.

Functional Testing:

Cas12a Nuclease functional testing was done by *in vitro* DNA cleavage assay with the following protocol, which gives more than 50% digestion of the substrate DNA as determined by agarose gel electrophoresis and densitometry (Fig. 3).

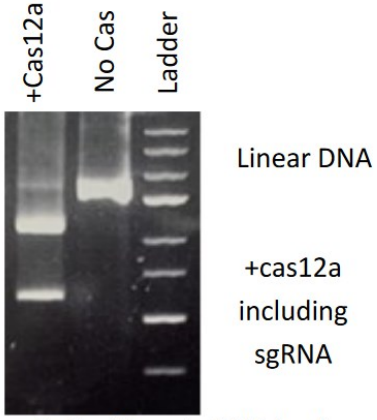


Figure 3. Cas12a cuts DNA in vitro (83% by densitometry)

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 Cas12a Reaction Buffer	3.0 µl
Cas12a Nuclease	1.0 µl (~160 ng)
Add H2O 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 DNA gel; for example use 1% agarose TBE gel.

   0.5x TBE= 0.54% Tris—0.28% Boric Acid—0.05% sodium-EDTA (w/v)

Related Products:

- Cas9 Nuclease (Cat.# 3273 & Cat.# 327b)
- Cas9 sgRNA synthesis kit
- Cas12a sgRNA synthesis kit
- sgRNA purification kit
- *Taq* DNA Polymerase (Cat.# 3243)
- *Taq* DNA Polymerase 2x Premix (Cat.# 3249)
- T4 DNA Ligase (Cat.# 3212)
- ig® 10B Chemically Competent Cells (Cat.# 1011-12)

Ordering Information:

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- Order by email, phone, or fax.
  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 **|** Toll-free : 855-835-7172 **|** Fax: (314) 942-3656
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References:

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. Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., Volz, S.E., Joung, J., van der Oost, J., Regev, A., Koonin, E.V., Zhang, F., (2015). Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. Cell 163, 759–771.

3. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. (2013) RNA-guided human genome engineering via Cas12a. Science. Feb 15;339(6121):823-6.

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