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DATASHEET

GenCRISPR™ LbuCas13a Nuclease

Cat. No.: Z03742

Product Introduction

GenCRISPR™ LbuCas13a Nuclease is an RNA-guided RNA endonuclease from *Leptotrichia buccalis*. Cas13a (previously known as C2c2) belongs to the Class 2 Type VI CRISPR-Cas system and contains a single protein effector with two HEPN domains. Cas13a exhibits two distinct ribonuclease activities. Cas13a possesses sequence-specific RNA cleavage activity *in vitro* and *in vivo*. And after specific cleavage, Cas13a activates collateral cleavage activity towards adjacent non-specific ssRNA sequences. The two distinct ribonuclease activities of Cas13a enable guide-RNA processing and RNA detection. The RNA detection empowered with Cas13a-crRNA complex and quenched fluorescent ssRNA as reporter has been reported to be versatile, rapid and high-specific.

Source: Recombinant Cas13a expressed by *E.coli*
Species: *Leptotrichia buccalis*
Accession#: C7NBY4
Tag: Tag-free
Apparent Molecular Weight: ~140 kDa, on SDS-PAGE under non-reducing conditions
Concentration: Please refer to the COA for the specific lot.
Storage Buffer: 10 mM Tris-HCl, 300 mM NaCl, 1.0 mM DTT, 50% glycerol, pH 8.0.
Storage& Stability: Store at -20 °C for up to 12 months from the date of manufacture. Avoid repeated freeze-thaw cycles. **Do not store below -20 °C!**
Application: crRNA-dependent RNA cleavage

Quality Control Specifications

Assay	Specifications
Appearance	Clear, colorless liquid
Purity by SDS-PAGE	≥ 90%
Activity	≥ 85% by *in vitro* digestion of ssRNA
Concentration	4 mg/ml±10% as analyzed by A280
Residual DNase	Undetectable
Residual RNase	Undetectable
Endotoxin Level	< 0.2 EU/μg

Reagents Supplied

Components	Size			Storage
GenCRISPR™ LbuCas13a Nuclease	100 µg	500 µg	1 mg	-20 °C
10 × Cas13a Reaction Buffer*	1.5 ml	1.5 ml	1.5 ml	-20 °C

* The reaction buffer is intended for Cas13a/crRNA-based Fluorescent Assays described in this manual, and not intended for other applications.

Protocol for Cas13a/crRNA-based Fluorescent Assays

1. **Reagent preparation**
   1) Synthesize specific crRNA; we recommend GenScript's CRISPR Synthetic Guide RNA Services (**see www.genscript.com)** for crRNA synthesis.
   2) Synthesize specific single-strand RNA (ssRNA) as substrate for cleavage; we recommend custom RNA synthesis using GenScript's RNA Synthesis Service (**see www.genscript.com**).
   3) Synthesize quenched fluorescent ssRNA; we recommend custom RNA synthesis using GenScript RNA Synthesis Service (**see www.genscript.com**).
   4) Prepare crRNA working solution at an appropriate concentration (e.g., 7.5 ng/µl) by diluting the stock solution with DEPC-Treated Water (nuclease-free) on ice.
   5) Prepare specific ssRNA working solution at an appropriate concentration (e.g., 5 ng/µl) by diluting the stock solution with DEPC-Treated Water (nuclease-free) on ice.
   6) The reaction volume is recommended to be set at 50 µl, and the reaction conditions should be optimized according to your specific applications.

2. **Assay procedure**
   1) Assemble the reaction in a nuclease-free PCR strip tube for step 1 at room temperature on a clean bench as the following order in the table.
   2) Assemble the reaction in a black ELISA Plate for step 2 at room temperature on a clean bench in the order indicated in the table.

Step 1: Prepare the Cas13a/crRNA complex	
Components	**Volume**
10 × Cas13a Reaction Buffer	1 µl
15 ng crRNA	2 µl (7.5 ng/µl)
200 ng GenCRISPR™ LbuCas13a Nuclease (Z03742)	2 µl (100 ng/µl)
DEPC-Treated Water (nuclease-free)	5 µl
Mix thoroughly, incubate for 10 min at 37 °C in a PCR instrument.	
Step 2: Fluorescent Assay	
Components	**Volume**
10 ng ssRNA	2 µl (5 ng/µl)
10 × Cas13a Reaction Buffer	4 µl
DEPC-Treated Water (nuclease-free)	29 µl
Cas13a/crRNA complex (from step 1)	10 µl
10 pmol Fluorescent RNA reporter	5 µl
Mix thoroughly, incubate for 30 min at 37 °C in an incubator. After incubation, read the	

fluorescence intensity (Excitation wavelength: 494 nm; Emission wavelength: 518 nm) using a microplate reader.

References:

1. Abudayyeh, Omar O., et al. "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector." *Science* 353.6299 (2016).
2. Gootenberg, Jonathan S., et al. "Nucleic acid detection with CRISPR-Cas13a/C2c2." *Science* 356.6336 (2017): 438-442.
3. Gootenberg, Jonathan S., et al. "Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6." *Science* 360.6387 (2018): 439-444.
4. Iwasaki, Roman S., and Robert T. Batey. "SPRINT: a Cas13a-based platform for detection of small molecules." *Nucleic acids research* 48.17 (2020): e101-e101.
5. East-Seletsky, Alexandra, et al. "Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection." *Nature* 538.7624 (2016): 270-273.

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