LwaCas13a (C2c2) Nuclease

#32117-EN Version e117.2.1

Description

LwaCas13a (previously known as LwaC2c2) is a crRNA-mediated RNA endonuclease derived from *Leptotrichia wadeim*. It can specifically recognize and cleave the RNA target with the PFS sequence. In addition, Cas13a also has the trans-cleavage activity, that is, when LwaCas13a binds crRNA and RNA target to form a ternary complex, it releases the trans-cleavage activity of Cas13a against non-target ssRNA sequences. This activity has also been applied in the development of rapid nucleic acids detection systems. For example, Prof. Feng Zhang's team from the Broad Institute employed the Cas13 trans-cleavage activities and successfully developed the "SHERLOCK" platform (for details, please refer to PMID: 28408723).

Product Components

Components	32117-01 (100 pmol)	32117-03 (1,000 pmol)
LwaCas13a Nuclease (10 μM) [a]	100 pmol	1,000 pmol
10 × HOLMES Buffer 2	1 mL	1 mL

a. The concentration of LwaCas13a Nuclease is 10 μM (10 pmol/μL);

Storage Conditions

Store at -20℃; transport at ≤0℃.

Experiment Procedure

1. *Trans*-cleavage experiment

Components	Volume	Final concentration
10 × HOLMES Buffer 2	2 μL	1 ×
10 μM LwaCas13a Nuclease	0.05~0.5 μL	25~250 nM
10 μM crRNA	0.05~0.5 μL	25~250 nM
10 μM Target RNA	0.05~0.5 μL	25~250 nM
10 μM ssRNA Reporter (FAM-BHQ1)	0.05~0.5 μL	25~250 nM
Nuclease-free Water	Up to 20 μL	

- *The amount of each component in the trans-cleavage system can be adjusted according to different experimental purposes. When the amount is small, each component can be diluted first and then added to the system. LwaCas13a Nuclease can be diluted with 1×HOLMES Buffer 2, and it must be used immediately after dilution (If you want to store the diluted Cas13 enzyme for a long term, please use Cas13 Dilution Buffer, #32013). crRNA, Target RNA and ssRNA Reporter can be diluted with Nuclease-free Water, but for extremely low concentrations of Target RNA (such as LOD experiments), it is recommended to dilute with 0.1 % Tween 20 and use low adsorption centrifuge tubes and tips etc.*
- *For the amount of probe used in the trans-cleavage system and the expected reaction time to reach the plateau, please refer to the specific value of transU of each batch of Cas enzyme. For details, please refer to the COA test report provided by Tolo Biotech.*

The real-time fluorescent quantitative PCR instrument detects the fluorescent signal from reaction at 37°C, and collects the fluorescent signal every 30 seconds.

Experimental Results

The tested trans-cleavage system in this experiment contained LwaCas13a, crRNA, ssRNA reporters, and RNA target of different concentrations. The reporters were labeled with a fluorophore (FAM) at the 5'-end and a quencher (BHQ1) at the 3'-end. The results showed LwaCas13a released the trans-cleavage activity against ssRNA and cleaved the ssRNA reporters to emit fluorescent signals at the presence of RNA target. The higher concentrations of the RNA target, the faster fluorescence signals produced, and the shorter time to reach the plateau.

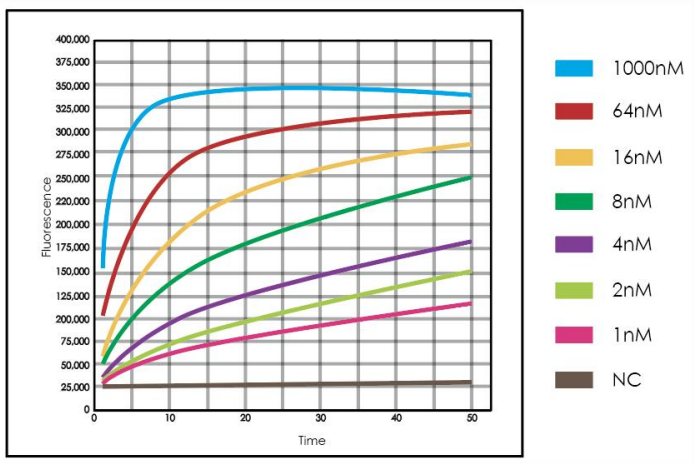

Fig 1. Results of Cas13 trans-cleavage at different concentrations of ssRNA target.