

## AapCas12b (C2c1) Nuclease (Lyophilized)

#V32118E / V32118T / V32118S

Version e152.3.2

### ■ Introduction

AapCas12b (previously known as C2c1) is a DNA endonuclease mediated by tracrRNA and crRNA (or sgRNA), which specifically cleaves the dsDNA target in a PAM (TTN)-dependent manner, generating DSBs with sticky ends, and cleave ssDNA target in a PAM-independent manner. In addition, both dsDNA and ssDNA targets can trigger the *trans*-cleavage activity of AapCas12b, that is, when the AapCas12b binds sgRNA and target DNA to form a ternary complex, the *trans*-cleavage activity of AapCas12b will be released to non-specifically cleave ssDNA sequences in the reaction system. AapCas12b is derived from *Alicyclobacillus acidophilus* and produced from *E. coli* BL21(DE3), with the optimal reaction temperature of 60 °C for *in vitro* analysis. Therefore, AapCas12b is more suitable for the creation of a “One-Step” CRISPR-Cas detection system when combined with the LAMP isothermal amplification technology.

This product is lyophilized glycerol-free AapCas12b nuclease, which can be shipped and stored at room temperature. After being dissolved with its reconstitution buffer, it shows the same performance as the non-lyophilized type of AapCas12b (C2c1) Nuclease (#32118, ToloBio). This product can be provided in a variety of lyophilized forms such as lyophilized powder and lyophilized beads to meet the needs of different downstream applications.

### ■ Components

AapCas12b Powder	V32118E-SP	V32118E-01	V32118E-02
○ AapCas12b Lyophilized Powder (SCREW CAP TUBES)	10 µg	100 µg	100 µg × 5
● Reconstitution Solution	200 µL	200 µL	1 mL
● 10 × HOLMES Buffer for Cas12b	1 mL	1 mL	1 mL × 5
<ul style="list-style-type: none"> <li>• 100 µg of AapCas12b nuclease = 750 pmol. It is recommended to use 150 µL of reconstitution solution to reconstitute 100 µg AapCas12b lyophilized powder, generating 5 µM (5 pmol/µL) liquid enzyme. Once dissolved, the liquid enzyme should be stored at -20 °C or below.</li> </ul>			
AapCas12b Powder (Single Reaction)	V32118T-SP	V32118T-01	V32118T-02
● AapCas12b Lyophilized Powder (8-TUBE STRIPS)	8 T	48 T	96 T
● 10 × HOLMES Buffer for Cas12b	1 mL	1 mL	1 mL
<ul style="list-style-type: none"> <li>• The amount of AapCas12b for a single reaction is 5 pmol and is recommended for a 20 µL CRISPR reaction system.</li> </ul>			
AapCas12b Beads (Single Reaction)	V32118S-SP	V32118S-01	V32118S-02
● AapCas12b Lyophilized Beads (8-TUBE STRIPS)	8 T	48 T	96 T
● 10 × HOLMES Buffer for Cas12b	1 mL	1 mL	1 mL
<ul style="list-style-type: none"> <li>• The amount of AapCas12b in a single lyophilized bead is 5 pmol and is recommended for a 20 µL CRISPR reaction system.</li> </ul>			

## ■ Storage

This product is stored and shipped at room temperature. After reconstitution, the liquid enzyme should be stored at -20 °C or below and is stable for at least one month. Repeated freezing and thawing should be avoided for reconstituted liquid enzyme.

## ■ Experimental Procedure

### AapCas12b (Cat # V32118E) CRISPR *Trans*-cleavage Experiment

1) Prepare the reaction mixture of CRISPR *Trans*-cleavage Experiment on ice as below.

Components	Volume	Final Concentration
10 × HOLMES Buffer for Cas12b (#32043)	2 μL	1 ×
5 μM AapCas12b (C2c1) Nuclease	1 μL	250 nM
10 μM sgRNA	0.5 μL	250 nM
1 μM Target DNA	0.5~5 μL	25~250 nM
10 μM HOLMES ssDNA Reporter (#31101)	0.5 μL	250 nM
Nuclease-free Water	Up to 20 μL	

2) Put the reaction mixture in the real-time PCR instrument to detect the fluorescent signal at 60 °C, and collect the fluorescent signals every 30 sec.

### AapCas12b (Cat# V32118T/V32118S) CRISPR *Trans*-cleavage Experiment

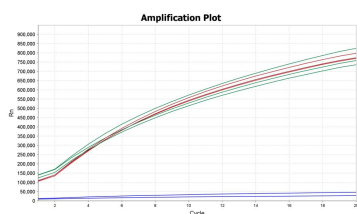
1) Prepare the reaction mixture of CRISPR *Trans*-cleavage Experiment on ice as below.

Components	Volume	Final Concentration
10 × HOLMES Buffer for Cas12b (#32043)	2 μL	1 ×
10 μM sgRNA	0.5 μL	250 nM
1 μM Target DNA	0.5~5 μL	25~250 nM
10 μM HOLMES ssDNA Reporter (#31101)	0.5 μL	250 nM
Nuclease-free Water	Up to 20 μL	

2) Dissolve the lyophilized powder/bead for a single reaction using the 20 μL reaction mixture prepared above.

3) Put the reaction mixture in the real-time PCR instrument to detect the fluorescent signal at 60 °C, and collect the fluorescent signals every 30 sec.

## ■ Experimental Results



- AapCas12b (C2c1) Nuclease (Lyophilized)
- AapCas12b (C2c1) Nuclease (liquid form, #32118)