Cas12 (AapCas12b) Kit

Cat #: orb2648680 (manual)

Product Description

AapCas12b enzyme reaction kit consisting of enzyme, buffer, and optional reporter and/or guide RNA.

Components

Component Name	20 µg	100 µg
AapCas12, Active	20 µg	100 µg
Cas12 Reaction Buffer, 10X	1ml	2 x 1ml
Cas12 ssDNA Reporter (optional)	10 nmol	10 nmol
Cas12 Guide RNA (optional, custom)	1 vial	1 vial

Formulations

Enzyme	50 mM sodium phosphate, pH 7.5, 300mM NaCl, 1mM DTT, 10% glycerol
Buffer	100mM Tris-HCl, pH 8.0, 500mM NaCl, 100m MgCl2, 1mg/mL (0.1% w/v)
Reporter	Lyophilized powder
Guide RNA	Nuclease free water

Storage and Stability

Store kit at -70°C. To avoid repeated handling and multiple freeze/thaw cycles aliquot components into smaller quantities. Protect reporter component from light.

Scientific Background

CRISPR (clustered regularly interspaced short palindromic repeats) and CRISPR-associated (Cas) proteins constitute the adaptive immune system in bacteria (1-2). This system has been leveraged to create an exemplary gene detection and genome editing tool with applications in both basic research and therapeutics development (3). AapCas12b belongs to the type V CRISPR effector, CRISPRCas12b/C2c1. It has both cis and trans nuclease cleavage activity. Due to its trans cleavage activity and excellent thermostability, it can be used in a wide range of biochemical applications including gene detection, mammalian genome editing, and gene activation (4).

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Reaction Protocol

Other Materials Required

- Filter pipette tips
- Nuclease free water
- Nuclease free microcentrifuge tubes
- Half-area solid black 96-well plate
- Microplate sealing tape
- Fluorescent microplate reader
- dsDNA substrate

Step 1: Thaw the active enzyme on ice. Prepare 1X Reaction Buffer with nuclease free water. Reconstitute reporter in nuclease free water. Equilibrate the buffer, reporter, guide RNA, and dsDNA substrate to ambient temperature.

Step 2: Prepare the following working solutions with 1X Reaction Buffer:

- 4X final concentration of Active Cas12 enzyme
- 4X final concentration of guide RNA
- 2X final concentration of substrate/reporter mix

Step 3: In a half-area solid black 96-well plate, add the following components and pre-incubate at room temperature for 15 minutes;

Component 1. 10 µL of 4X Active Cas12

Component 2. 10 µL of 4X guide RNA

Note: A blank control can be set up as outlined in step 3 by replacing the enzyme working solution with an equal volume of the reaction buffer.

Step 4: To each assay well, add 20 µL of the 2X substrate/reporter mix. Shake the plate for 1 minute on a tabletop orbital shaker. Seal the assay wells with microplate sealing tape and incubate at 37°C for 10-30 minutes.

Step 5: Equilibrate the plate to ambient temperature and then remove the microplate sealing tape. Read fluorescence on a microplate reader.

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