# Cas12a, His-Tag (Acidaminococcus sp.) Recombinant

Catalog: 101627
Lot: 240314

## Product Information

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|---|---|
| **Description:** | Recombinant Acidaminococcus AsCas12a (CRISPR-associated endonuclease Cpf1). This construct contains a C-terminal His-tag. AsCas12a is a CRISPR-associated endonuclease engineered for combinatorial genetic screening with high efficiency. |
| **Species:** | Acidaminococcus |
| **Construct:** | Cas12a (Full Length-His) (Acidaminococcus) |
| **Concentration:** | 0.20 mg/ml |
| **Expression System:** | *E. coli* |
| **Purity:** | 75% |
| **Format:** | Aqueous buffer solution. |
| **Formulated In:** | 50 mM Tris, pH 7.5, 600 mM NaCl, 10% glycerol, and 2 mM DTT |
| **MW:** | 125 kDa |
| **Uniprot Number:** | U2UMQ6 |
| **Stability:** | At least 6 months at -80°C. |
| **Storage:** | -80°C |
| **Instructions for Use:** | Thaw on ice and gently mix prior to use. DO NOT VORTEX. Perform a quick spin before opening. Aliquot into small volumes and flash freeze for long term storage. Avoid multiple freeze/thaw cycles. |
| **Assay Conditions:** | Varying amounts of AsCas12a activity was measured using a CRISPR-based fluorescent reporter assay for optimal results. Target DNA cutting and indiscriminate single stranded DNA collateral cleavage was activated using RNA-guided DNA Binding to Cas12. Emission of fluorescent signal is due to the degradation of ssDNA reporters upon cleavage.<br>Active Cas12 was thawed on ice while 1X Endonuclease Buffer containing 10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 10 mM $MgCl_2$, and 0.1 mg/ml BSA, guide RNA (custom designed crRNA), ds DNA activator (complementary sequence to crRNA and a PAM sequence specific for Cas enzyme) and FQ-ssDNA substrate (labeled with fluorophore and a quencher) were equilibrated to room temperature. Next three working solutions of Active Cas12 (4X final concentration) guide RNA (4X final concentration) and activator/reporter mix containing ds DNA activator and ssDNA reporter (2X final concentration), were prepared using 1X Endonuclease Buffer. 10 µl of 4X active Cas12 and 10 µl of 4x guide RNA were then preincubated in half the area of a solid black 96-well plate for 10 minutes at room temperature. After preincubation, 20 µl of 2X activator/reporter mix was added to plate and placed on shaking incubator for 1 min. The plate was then sealed and incubated at 37°C for 10-30 minutes. Plate was then equilibrated to room temperature, plate sealer removed and fluorescence read on a microplate reader. Negative control was measured by replacing enzyme working solution with equal volume of assay buffer. |
| **Applications:** | Useful for DNA cleavage, targeted genome editing, the study of enzyme kinetics, screening inhibitors, and selectivity profiling. |

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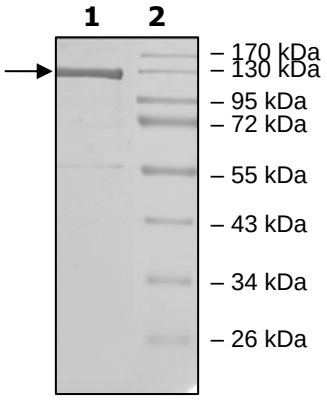
## Quality Control Data

### 4-20% SDS-PAGE Coomassie Staining




### AsCas12a Nuclease Activity