

AapCas12b, Active

Recombinant protein expressed in *E. coli* cells

Catalog # C12B1-E311U

Lot # 1K1614-1

Product Description

Recombinant full length, tag-free *Alicyclobacillus acidiphilus* AapCas12b protein expressed in *E. coli*.

Accession number: [WP_067623834.1](https://www.ncbi.nlm.nih.gov/nuccore/WP_067623834.1)

Alternative name (s)

c2c1, CRISPR-associated endonuclease C2c1, Cas12b

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.5, 300mM NaCl, 1mM DTT, 10% glycerol.

Storage and Stability

Store at -70°C. To avoid repeated handling and multiple freeze/thaw cycles aliquot product into smaller quantities.

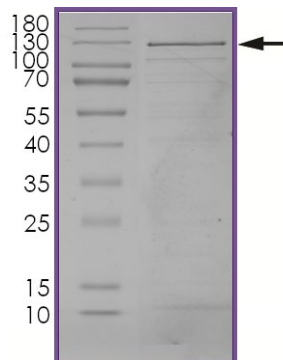
Scientific Background

CRISPR (clustered regularly interspaced short palindromic repeats) and their CRISPR-associated (Cas) proteins constitute the adaptive immune system in bacteria (1-2). This system has been leveraged to create an exemplary genome editing tool with applications in both basic research and therapeutics development (3). AapCas12b belongs to the type V CRISPR effector, CRISPR-Cas12b/C2c1, and due to its thermostability, can be used in a wide range of biomedical applications including loop-mediated isothermal amplification (LAMP), mammalian genome editing, and gene activation (4).

References

1. Horvath, P., et al. 2010. CRISPR/Cas, the immune system of bacteria and archaea. *Science*, 327(5962):167-170.
2. Morange, M. 2015. What history tells us XXXVII. CRISPR-Cas: The discovery of an immune system in prokaryotes. *J. Biosci.* 40(2):221-223.
3. Gier, R.A., et al. 2020. High-performance CRISPR-Cas12a genome editing for combinatorial genetic screening. *Nat. Commun.* 11(1):1-9.
4. Teng F, et al. 2018. Repurposing CRISPR-Cas12b for mammalian genome engineering. *Cell discovery*. 4(1):1-5.

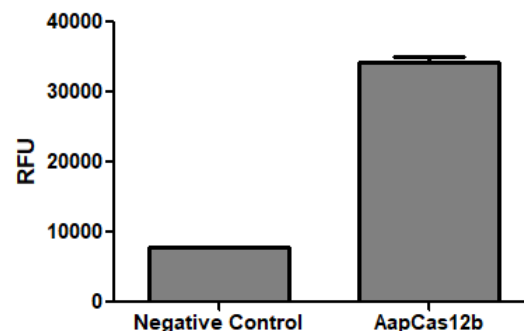
Purity



SDS-PAGE gel image

The purity of AapCas12b, Active was determined to be >80% by densitometry. Calculated MW: 130.5 kDa
Observed MW: ~128 kDa

Activity



AapCas12b nuclease activity assayed using a fluorogenic substrate and 40nM of enzyme as per the Cas12 Activity Assay Protocol provided below.

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Purity >80%

Concentration 0.1 µg/µl
7.66 pmol/µg

Stability 1yr at -70°C from date of shipment

Storage & Shipping Store at -70°C. To avoid repeated handling and multiple freeze/thaw cycles aliquot product into smaller quantities.
Product shipped on dry ice.

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Cas12 Activity Assay Protocol

Reaction Components

Active DNA Endonuclease

It is recommended that the researcher perform a serial dilution of Active Cas12 diluted with 1X Endonuclease Buffer for optimal results.

Endonuclease Buffer (5x)

Buffer components: 50 mM Tris-HCl pH8.0, 250 mM NaCl, 50 mM MgCl₂, 0.5 mg/mL BSA.

CRISPR RNA

Single guide RNA containing a custom-designed crRNA fused a scaffold sequence responsible for recruiting the Cas enzyme.

Double-stranded DNA (dsDNA) activator

A dsDNA oligo that contains the complementary sequence to the crRNA and a PAM sequence specific for the Cas enzyme.

Reporter Substrate

A short single-stranded DNA labeled with a fluorophore and a quencher on the two termini.

Assay Protocol

The DNA trans-cleavage activity of Cas12 is detected in a CRISPR-based fluorescent reporter assay. RNA-guided DNA binding to Cas12 activates the enzyme, induces target DNA cutting as well as indiscriminate single-stranded DNA collateral cleavage activity. The latter leads to the degradation of ssDNA reporters that, emits a fluorescent signal on cleavage.

- Step 1.** Thaw the active Cas12 on ice. Prepare 1X Endonuclease Buffer. Equilibrate the buffer, guide RNA, dsDNA activator and FQ-ssDNA substrate to ambient temperature.
- Step 2.** Prepare the following working solutions with 1X Endonuclease Buffer:
 - 4X final concentration of Active Cas12
 - 4X final concentration of guide RNA
 - 2X final concentration of activator/reporter mix containing dsDNA activator and ssDNA reporter.
- 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 assay buffer.
- Step 4.** To each assay well, add 20 µL of the 2X activator/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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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: AapCas12b, Active**Catalog # C12B1-E311U**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Supplier of Datasheet: SignalChem Diagnostics Inc.
Street Address: 190-13160 Vanier Place
City, Prov. Postal Code: Richmond, BC, V6V 2J2
Country: Canada
Emergency Phone: 1-888-606-3424 (Toll free)
1-778-326-0223 (local)

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Not classified.
- **Hazard Pictograms:** None.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** None.
- **Other hazards:** None known.

Article 3 - Composition/Information on Ingredients

Chemical Characterization: Mixture.

Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	10%
NaCl	Sodium chloride	7647-14-5	1.75%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
Protein	N/A	N/A	<0.1%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0154%

Article 4 - First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If casualty cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Consult a physician.
- **After swallowing:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store according to product label instructions. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
NA
- **Appropriate engineering controls:**
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.
Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.
Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	Danger of explosion: Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	Explosion limits: Not available.
pH: Not available.	Decomposition temperature: Not available.
Melting point/freezing point: Not determined.	Vapor pressure at 20 °C: Not available.
Boiling point/Boiling range: Not determined.	Density: Not determined.
Flash point: Not determined.	Relative density: Not determined.
Flammability (solid, gaseous): Not determined.	Vapor density: Not determined.
Ignition temperature: Not determined.	Evaporation rate: Not determined.
Auto-igniting: Product is not self-igniting.	Solubility in / Miscibility with Water: Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Not determined.
- **Hazardous decomposition products:** Not determined.

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Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
 - Inhalation: No data available
 - Ingestion: No data available
 - Skin: No data available
 - Eyes: No data available
- **Signs and Symptoms of Exposure:** No data available
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 - Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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