SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
C12CR-E241G-05	5 µg
C12CR-E241G-10	10 µg
C12CR-E241G-20	20 ug
C12CR-E241G-50	50 ug

Cas12 (LbCpf1), Active

Recombinant protein expressed in E. coli cells

Catalog # C12CR-E241G

Lot # N3617-1

Product Description

Recombinant Lachnospiraceae Cas12 (LbCpf1) (1-1227) was expressed in E. coli with a N-terminal GST tag. The protein accession number is ATB19154.

Gene Aliases

Cpf1 (synthetic construct)

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 1mM DTT, 10% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

CRISPR (clustered regularly interspaced short palindromic repeat) and their CRISPR-associated (Cas) proteins constitute the adaptive immune system in bacteria (1-2). This system has been redesigned to create an exemplary genome editing tool for application to RNA-based therapeutics development (3). Developing rapid and sensitive diagnostic tools is key for successful patient management and curbing the disease spread. Several CRISPR-Cas12 based SARS-CoV-2 detection assays have been developed (4,5). CRISPR/Cas12 (LbCpf1) is a Cas12 synthetic construct derived from Lachnospiraceae bacterium.

References

1. Horvath, P., et al. CRISPR/Cas, the immune system of bacteria and archaea. Science, 327(5962):167-170.
2. Morange, M. What history tells us XXXVII. CRISPR-Cas: The disconvery of an immune system in prokaryotes. J. Biosci. 40(2):221-223.
3. Gier, R.A., et al. High-performance CRISPR-Cas12a genome editing for combinatorial genetic screening. Nat. Commun. 2020, 11(1):1-9.
4. Broughton JP, et al: Rapid detection of 2019 novel coronavirus SARS-CoV-2 using a CRISPR-based DETECTR lateral flow assay. medRxiv doi: https://doi.org/10.1101/ 2020.03.06.20032334.
5. Lucia C, et al: An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12. bioRxiv. doi: https://doi.org/10.1101/ 2020.02.29.971127.

Specific Activity

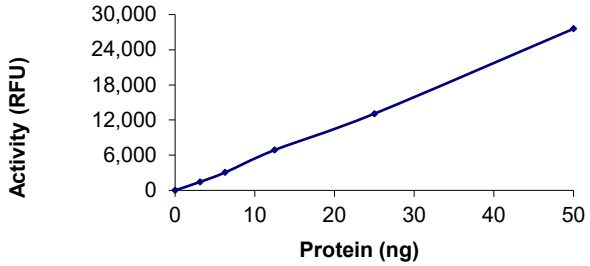

Figure 1. The specific activity of Cas12 (LbCpf1) was determined to be **11.4 nmol/min/mg** as per activity assay protocol.

Purity

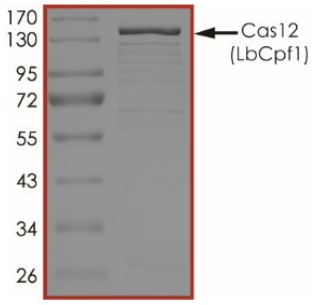

Figure 2. The purity of Cas12 (LbCpf1) was determined to be **~70%** by densitometry, approx. MW **150 kDa** (calculated MW ~164 kDa).

Cas12 (LbCpf1), Active

Recombinant protein expressed in E. coli cells

Catalog #	C12CR-E241G
Specific Activity	11.4 nmol/min/mg
Lot #	N3617-1
Purity	~70%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Activity Assay Protocol

Reaction Components

Active DNA Endonuclease (Catalog#:C12CR-E241G)

Active Cas12a diluted with Endonuclease Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active Cas12a for optimal results).

Endonuclease Buffer (5x)

Buffer components: 50 mM Tris-HCl pH8.0, 250 mM NaCl, 50 mM $MgCl_2$, 0.5 mg/mL BSA.

CRISPR RNA

Guide RNA targeting a SARS-CoV-2 RdRp gene fragment, reconstituted in nuclease-free water to a working stock of 100 μM.

Double-stranded DNA (dsDNA) activator

A SARS-CoV-2 RdRp gene fragment, amplified by PCR using RdRp expression plasmid.

Substrate

A short single-stranded DNA labeled with a fluorophore and a quencher (FQ-ssDNA), reconstituted in nuclease-free water to a working stock of 100 μM.

Assay Protocol

The DNA trans-cleavage activity of Cas12a is detected in a CRISPR-based fluorescent reporter assay. RNA-guided DNA binding to Cas12a 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. Generate the dsDNA activator by PCR using SARS-CoV-2 RdRp expression plasmid as template, and appropriate primers for the intended gene fragment.

Step 2. Thaw the active Cas12a on ice. Prepare 1X Endonuclease Buffer. Equilibrate the buffer, guide RNA, dsDNA activator and FQ-ssDNA substrate to ambient temperature.

Step 3. Prepare the following working solutions with 1X Endonuclease Buffer:

- 4X final concentration of Active Cas12a (Catalog # C12CR-E241G)
- 4X final concentration of guide RNA (400 nM)
- 2X activator/reporter mix containing 45 nM RdRp gene fragment and 5 μM FQ-ssDNA.

Step 4. In a half-area solid black 96-well plate, add the following components and pre-incubate at room temperature for 10 minutes;

Component 1. 10 μL of 4X Active Cas12a

Component 2. 10 μL of 4X guide RNA

Note: A blank control can be set up as outlined in step 4 by replacing the enzyme working solution with an equal volume of the assay buffer.

Step 5. 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 a plate sealer and incubate at 37°C for 30-45 minutes.

Step 6. Equilibrate the plate to ambient temperature and then remove the plate sealer. To determine enzyme specific activity, a series of 6-carboxyfluorescein (6-FAM) standard solutions, supplemented with the guide RNA, are also dispensed to the plate.

Step 7. Read the plate on a fluorimeter using excitation/emission wavelengths of 485/535 nm.

Step 8. Using the 6-FAM standard curve, determine the concentration of cleaved DNA reporter (μM) and calculate the specific activity as outlined below.

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[ssDNA](\mu M) \times Reaction\ Volume(\mu L) \times 10^{-3}}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)}$$

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: Cas12 (LbCpf1), Active **Catalog # C12CR-E241G**

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.

Manufacturer's Name:	SignalChem Biotech Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixtures.
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	0.8766%
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 – Amino – 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	0.788%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	0.010%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0154%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If cannot breath, 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. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

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.

SAFETY DATA SHEET

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 sate handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m³ for mist	British Columbia, Canada
TWA	3 mg/m³ for respirable mist	British Columbia, Canada
TWA	10 mg/m³	Alberta, Canada
TWAEV	10 mg/m³	Ontario, Canada
TWAEV	10 mg/m³	Quebec, Canada
TWA	10 mg/m³	USA

- **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:** Lower: 0.9 Vol %; Upper: 0.0 Vol %.
pH: Not available.	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** 0.1 hPa
Boiling point/Boiling range: 100 °C.	**Density:** Not determined.
Flash point: > 100 °C.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: 400 °C.	**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:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

SAFETY DATA SHEET

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: May be harmful if inhaled. May cause respiratory tract irritation.
  Ingestion: May be harmful if swallowed.
  Skin: May be harmful if absorbed through skin. May cause skin irritation.
  Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
  Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** Not applicable.
- **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.