



Catalog # Aliquot Size

C19PL-G241H-05 5 µg
C19PL-G241H -10 10 µg

2019-nCoV PLpro, Active

Recombinant viral protein expressed in *E. coli* cells

Catalog # C19PL-G241H

Lot # N3593-12

Product Description

Recombinant full-length 2019-nCoV PLpro/papain-like protease, part of a large replicase polyprotein 1ab (E1564-Y1882), was expressed in *E. coli* cells with a C-terminal His tag. The gene accession number of polyprotein 1ab is [QHD43415](#).

Alternative name(s)

SARS-CoV-2 PLpro. The Uniprot accession number is [P0DTD1](#).

Concentration

0.2 µg/µl

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.5, 300mM NaCl, 150mM imidazole, 1mM DTT, 10% glycerol.

Storage and Stability

Aliquot product into smaller quantities after centrifugation and store at -70°C. Avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

2019-nCoV papain-like protease (PLpro), also known as SARS-CoV-2 PLpro, is a virally encoded cysteine protease that acts on three cleavage sites of the viral polyprotein to release mature non-structural proteins (nsp) 1, 2 and 3, a process that is essential for viral replication. In addition, PLpro possesses deubiquitinating and deISGylating activities, thus negatively regulating the ubiquitin and ISG15-dependent host immune response during viral infection. PLpro also participates together with nsp4 in the assembly of virally-induced cytoplasmic double-membrane vesicles necessary for viral replication.

References

1. Wu F. et al: A new coronavirus associated with human respiratory disease in China. *Nature* 579: 265-269, 2020
2. Báez-Santos Y. M. et al: The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. *Antiviral Res.* 115: 21-38, 2015
3. Lindner H. A. et al: The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. *J Virol.* 79:15199-15208, 2005

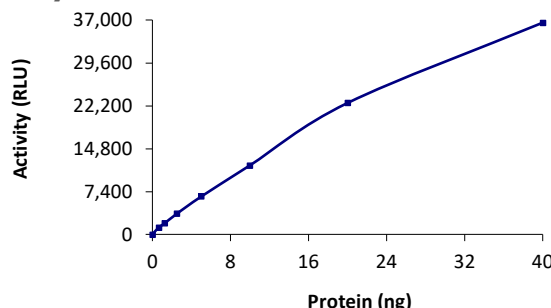
Purity



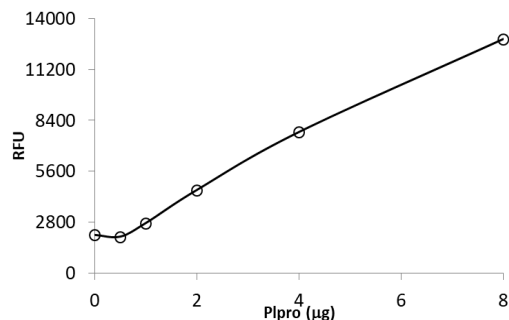
SDS-PAGE gel image

The purity of 2019-nCoV PLpro was determined to be ~90% by densitometry. Approx. MW 38 kDa.

Activity



The DUB specific activity of 2019-nCoV PLpro was determined to be **7.2 nmol/min/mg** as per the DUB activity assay protocol. The IC₅₀ of the reversible DUB inhibitor PR-619 was **10.13 µM** in the PLpro inhibition profile assay.



The protease specific activity of 2019-nCoV PLpro was determined to be **≥450 pmol/min/mg** at an enzyme concentration of **4 µg** as per the proteolytic activity assay protocol.

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Deubiquitinase Activity Assay Protocol

Reaction Components

Active Enzyme (Catalog #: C19PL-G241H)

Active 2019-nCoV PLpro (Catalog #: C19PL-G241H) diluted with DUB Reaction 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 2019-nCoV PLpro for optimal results).

Luciferin Detection Reagent (Promega, Cat # V8920)

Luciferin Detection Reagent
Reconstitution Buffer

4X DUB Reaction Buffer (Catalog # D01-09)

Buffer components: 0.2M HEPES (pH7.5), 2mM EDTA, 4mg/ml BSA, add 0.4mM DTT in 4X Buffer prior to use, 0.1mM DTT final assay concentration.

DUB Substrate I (Catalog # U06-57)

Recombinant human ubiquitin-based proluciferin substrate with a final concentration of 1mg/ml.

Assay Procedure

The 2019-nCoV PLpro deubiquitinase activity is detected using a ubiquitin-based proluciferin substrate that is processed through coupled-enzyme reactions to release a light signal. The light intensity is quantitatively correlated with the enzyme activity.

Step 1. Thaw the Active 2019-nCoV PLpro, DUB Substrate I and reaction buffer on ice. Thaw reconstituted Luciferin Detection Reagent at room temperature and keep away from light.

Step 2. Prepare 1X DUB Reaction Buffer containing 10 mM DTT; prepare enzyme and substrate working stock solutions with the buffer.

Step 3. In a half-area solid white 96-well plate, add the following components to bring the reaction volume to 20 µl:

Component 1. 10 µl of 1X DUB Reaction Buffer (replace with test compound solution if performing HTS assay)

Component 2. 5 µl of Active 2019-nCoV PLpro working solution (for HTS assay, pre-incubate for 15 minutes before adding substrate)

Component 3. 5 µl of 2.4 µM substrate working solution

Note 1: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of reaction buffer.

Note 2: A series of aminoluciferin (AML) standard solutions can be included with the enzyme assay in order to determine the specific activity of the enzyme.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Incubate the plate at room temperature for 30 minutes.

Step 5. Add 20 µl of Luciferin Detection Reagent to all wells, mix by shaking for 2 minutes. Incubate the plate at room temperature for 30 minutes.

Step 6. Read the plate using the KinaseGlo luminescence protocol on a GloMax plate reader (Promega, Cat# E7031)

Step 7. Using an AML standard curve to determine the concentration of AML produced (µM) and calculate the enzyme specific activity as outlined below.

$$\text{Enzyme Specific Activity (SA) (nmol/min/mg)} \\ = \frac{[AML](\mu\text{M}) \times \text{Reaction Volume}(\mu\text{l})}{\text{Reaction Time (min)} \times \text{Enzyme Amount (mg)}} \times 10^{-3}$$

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Proteolytic Activity Assay Protocol

Reaction Components

Active Protease (Catalog #: C19PL-G241H)

Active PLpro diluted with PLpro Cleavage 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 PLpro for optimal results).

PLpro Cleavage Buffer

Buffer components: 50 mM HEPES, pH 7.5. Add fresh DTT (SignalChem, Catalog #: D86-09B-10) to 1 mM prior to use.

Substrate

Synthetic FRET peptide substrate specific for PLpro was reconstituted in Milli-Q water to a working stock of 500 μ M.

Assay Protocol

The PLpro protease activity is detected in a FRET-based assay using a Edans/Dabcyl peptide substrate. In the intact FRET peptide, the fluorescence of Edans is quenched due its close proximity to the Dabcyl quencher. Upon cleavage into two separate fragments by the protease, the fluorescence is recovered, and can be monitored at excitation/emission wavelengths = 340 nm/495 nm.

Step 1. Thaw the active PLpro (C19PL-G241H) on ice. Equilibrate the Cleavage Buffer and substrate stock solution to ambient temperature.

Step 2. Prepare the following working solutions with PLpro Cleavage Buffer:

- o 2X final concentration of Active PLpro (Catalog # C19PL-G241H)
- o 2X FRET peptide solution (40 μ M)

Step 3. In a half-area solid black 96-well plate, add the following components to bring the reaction volume to 50 μ l:

Component 1. 25 μ l of 2X Active PLpro

Component 2. 25 μ l of 2X FRET peptide

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. Mix 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 minutes.

Note: A series of free Edans standard solutions, supplemented with the FRET substrate, can be included at the end of the incubation period in order to determine the specific activity of the enzyme.

Step 5. Equilibrate the plate to ambient temperature and then remove the plate sealer.

Step 6. Read the plate on a fluorimeter using Ex/Em = 340/495 nm.

Step 7. Using the Edans standard curve, determine the concentration of the Edans-peptide fragment produced (μ M) and calculate the enzyme specific activity as outlined below.

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

$$= \frac{[Edans](\mu M) \times Reaction Volume(\mu l)}{Reaction Time (min) \times Enzyme Amount (mg)}$$

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: 2019-nCoV PLpro, Active**Catalog # C19PL-G241H**

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:** None.
- **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%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	0.7098%
Protein	N/A	No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.00154%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If 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 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:**
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: >100 °C.	Density: Not determined.
Flash point: > 100 °C.	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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