Catalog #	Aliquot Size
C19CL-G242H-05	5 µg
C19CL-G242H-10	10 µg
C19CL-G242H-20	20 µg
C19CL-G242H-50	50 µg

2019-nCoV 3CLpro, Active

Recombinant SARS-CoV-2 protein expressed in *E. coli* cells

Catalog # C19CL-G242H

Lot # F3832-4

Product Description

Recombinant 2019-nCoV 3CLpro, part of a large replicase polyprotein 1ab (S3264-Q3569), was expressed in *E. coli* cells with an N-terminal His tag. The gene accession number of polyprotein 1ab is QHD43415.

Alternative name(s)

SARS-CoV-2 3CLpro; NSP5; Mpro

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.5, 300mM NaCl, 200mM imidazole, 1 mM 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.

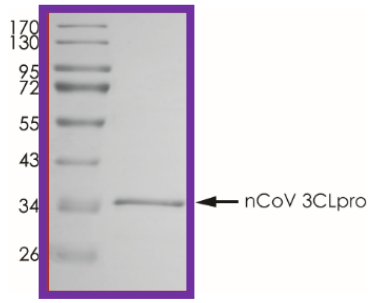
Scientific Background

SARS-CoV-2 3C-like proteinase (3CLpro) is the nonstructural protein number 5, also a cysteine protease that is released from the virally encoded polyprotein by extensive proteolytic processing. The 3CLpro cleaves the C-terminus of the large replicase polyprotein 1ab (~790 kDa) at no fewer than 11 sites (1). Inhibiting the activity of this enzyme would block viral replication (2). The functional importance of 3CLpro in the viral life cycle makes this protease an attractive target for the development of drugs directed against SARS and other coronavirus infections (3).

References

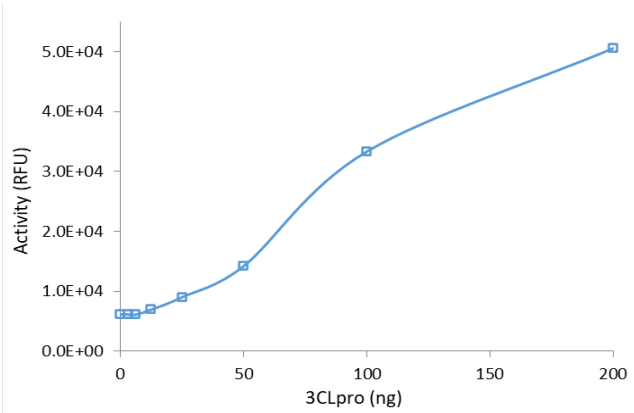
1. Hegyi, A. *et al*: Conservation of substrate specificities among coronavirus main proteases. J Gen Virol. 2002,83:595-599.
2. Zhang L. *et al*: Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science. 2020, 368:409-412.
3. Anand K. *et al*: Coronavirus main proteinase (3CLpro) structure: Basis for design of anti-SARS drugs. Science. 2003, 300:1763-1767.

Purity



SDS-PAGE gel image
The purity was determined to be **>90%** by densitometry.
Approx. MW **35 kDa**.

Activity



The protease specific activity of 3CLpro was determined to be **238 nmol/min/mg** at an enzyme concentration of **58 nM** (100ng) as per the activity assay protocol.

2019-nCoV 3CLpro, Active

Recombinant SARS-CoV-2 protein expressed in *E. coli* cells

Catalog #	C19CL-G242H
Specific Activity	238 nmol/min/mg
Lot #	F3832-4
Purity	>90%
Concentration	0.1µg/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.

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or by email: orders@signalchem.com or IVD@signalchem.com - www.signalchem.com

Activity Assay Protocol

Reaction Components

Active Protease (Catalog #: C19CL-G242H)

Active 3CLpro diluted with 3CLpro 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 3CLpro for optimal results).

3CLpro Cleavage Buffer

Buffer components: 20 mM Tris-HCl, pH 7.3, 100 mM NaCl, 1mM EDTA. Add fresh DTT (SignalChem, Catalog #: D86-09B) to 5 mM prior to use.

Substrate (Catalog #: CL01-58)

Synthetic 3CLpro FRET substrate was reconstituted in Milli-Q water to a working stock of 500 μM.

Assay Protocol

The 3CLpro protease activity is detected in a FRET-based assay using 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 an excitation/emission wavelengths of 340 nm/495 nm.

Step 1. Thaw the active 3CLpro (C19CL-G242H) on ice. Equilibrate the Cleavage Buffer and substrate stock solution to ambient temperature.

Step 2. Prepare the following working solutions with 3CLpro Cleavage Buffer:

- 2X final concentration of Active 3CLpro (C19CL-G242H)
- 2X FRET peptide substrate solution (40 μM)

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

Component 1. 25 μl of 2X Active 3CLpro (C19CL-G242H)

Component 2. 25 μl of 2X FRET peptide substrate

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 cleavage 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 ambient temperature for 60 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. 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 (μM) 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 3CLpro, Active **Catalog # C19CL-G242H**

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:** 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	≤1.36%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	≤0.7098%
Protein	N/A	N/A	≤0.02%
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:** 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 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:**
  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.