



Catalog # Aliquot Size

C19RP-G241H-10	10 µg
C19RP-G241H-20	20 µg
C19RP-G241H-50	50 µg
C19RP-G241H-100	100 µg

2019-nCoV RdRp, Active

Recombinant viral protein expressed in *E. coli* cells

Catalog # C19RP-G241H

Lot # S3415-10

Product Description

Recombinant 2019-nCoV RNA-dependent RNA polymerase (RdRp) was expressed in *E. coli* cells using a C-terminal His tag. The gene accession number is [QHD43415](#).

Alternative name(s)

nsp12

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.5, 300mM NaCl, 150mM imidazole, 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

A novel coronavirus (SARS-CoV-2) has caused a global COVID-19 pandemic of respiratory disease in 2020 (1). RNA-dependent RNA polymerase (RdRp) is an enzyme that is crucial to life cycle of RNA viruses including coronaviruses (2). RdRp catalyzes the synthesis of viral RNA, thereby playing a central role in the replication and transcription cycle of COVID-19 viruses, possibly in complex with nsp7 and nsp8 (3). Therefore, RdRp is a prime target for nucleotide analog antiviral inhibitors, such as remdesivir, for development of new antiviral therapeutics (4).

References

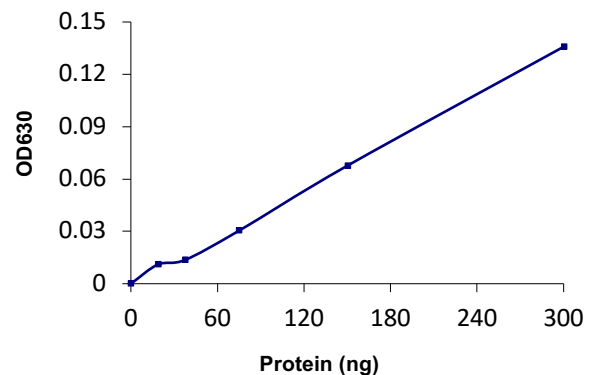
1. Zhou P, et al: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020, 579:270-89
2. Subissi L, et al: One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. *Proc. Natl. Acad. Sci. U.S.A.* 2014, 111: E3900-E3909.
3. Gao Y, et al: Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*. 2020, 368: 779-782.
4. Wang M, et al: Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 2020, 30:269-271.

Purity



SDS-PAGE gel image
The purity of 2019-nCoV RdRp was determined to be **>75%** by densitometry.
Approx. MW **100 kDa**.

Activity



The specific activity of 2019-nCoV RdRp was determined to be **9.3 nmol/min/mg** as per the activity assay protocol.

2019-nCoV RdRp, Active

Recombinant viral protein expressed in *E. coli* cells

Catalog #	C19RP-G241H
Specific Activity	9.3 nmol/min/mg
Lot #	S3415-10
Purity	>75%
Concentration	0.05 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.

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Activity Assay Protocol

Reaction Components

2019-nCoV RdRp, Active (Catalog: C19RP-G241H)

Active RdRp diluted with RNA Polymerase 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 RdRp for optimal results).

RNA Polymerase Buffer

Buffer components: 50 mM HEPES, pH 8.0, 2.5 mM MgCl₂. Add 4 mM DTT (SignalChem, Catalog #: D86-09B-10) and 0.4 unit/μl RNasin Plus (Promega) prior to use.

Substrate

Template/primer: poly-rC and oligo G₁₂ (Eurofins Genomics) dissolved in RNase-free water to 200 μM working stocks

rGTP, 10 mM (Promega)

Detection Reagents

Inorganic Pyrophosphatase, 1 mg/ml (Millipore Sigma)

Sodium pyrophosphate (Millipore Sigma)

BIOMOL® GREEN Reagent (Enzo Life Sciences)

Assay Protocol

The RdRp polymerase activity is detected in a coupled-enzyme assay wherein the pyrophosphate generated from the polymerase reaction is subsequently broken down by a pyrophosphatase, producing two molecules of monophosphate that form a colored complex with the BIOMOL® GREEN Reagent. The amount of complex can be then measured by standard UV spectrometers.

- Step 1.** Thaw the active RdRp on ice. Equilibrate the RNA Polymerase Buffer and all assay reagents to ambient temperature.
- Step 2.** Prepare the following working solutions with the RNA Polymerase Buffer:
 - 2.5X final concentration of Active RdRp (Catalog # C19RP-G241H)
 - 2.5X Substrate Cocktail: 0.5 mM rGTP, 20 μg/ml poly-rC and 2 μg/ml oligo G₁₂
 - 5X final concentration, or 20 μg/ml of inorganic pyrophosphatase
- Step 3.** In a half-area clear bottom black 96-well plate, add the following components to bring the initial reaction volume to 25 μl:
 - Component 1.** 10 μl of 2.5X Substrate Cocktail
 - Component 2.** 10 μl of 2.5X Active RdRp
 - Component 3.** 5 μl of 5X pyrophosphatase

Note 1: A blank control can be set up as outlined in step 3 by replacing the enzyme working solution with an equal volume of Reaction Buffer.

Note 2: A series of pyrophosphate (PPi) 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. Seal the assay wells with a plate sealer and incubate at 37°C for 60 minutes.
- Step 5.** Equilibrate the plate to ambient temperature and then remove the plate sealer.
- Step 6.** Add 100 μl of BIOMOL® GREEN Reagent to all assay wells. Mix for 1 minute on an orbital shaker and then incubate at ambient temperature for 30 minutes.
- Step 7.** Read the plate on a UV-Vis spectrometer at 630nm.
- Step 8.** Using the PPi standard curve, determine the amount of PPi produced (nmol) and calculate the enzyme specific activity as outlined below.

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

$$= \frac{\text{nmol of PPi produced}}{\text{Reaction Time (min)} \times \text{Enzyme Amount (mg)}}$$

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: 2019-nCoV RdRp, Active

Catalog # C19RP-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.

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	≤1.02%
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 – Amino – 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	7782-85-6	0.3152%
Protein	N/A	N/A	≤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:** Breathe 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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