



2019-nCoV NSP14 Methyltransferase, Active

Recombinant protein expressed in *E. coli* cells

Catalog # C19NS-E311H

Lot # E4319-10

Product Description

Recombinant NSP14 (5925-6452) was expressed in *E. coli* using a C-terminal His tag. The gene accession number is [QHD43415](#).

Alternative name(s)

NSP14: Non-structural protein 14

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 best performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

Coronaviruses code for a bifunctional non-structural protein 14 (NSP14) that is important for viral replication and transcription. NSP14 contains an N-terminal exoribonuclease (ExoN) domain for proof reading during viral replication and a C-terminal N7 methyltransferase (N7-MTase) domain for mRNA capping. NSP14 associates with several viral proteins (1). It can bind to NSP10 and the resulting complex exhibits enhanced ExoN activity *in vitro* (2). It can also bind to the polymerase complex (NSP12/NSP8/NSP7) forming a complex that retains all associated enzymatic activities (3).

References

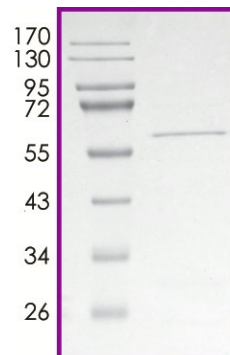
1. Ma, Y. et al: Structural basis and functional analysis of the SARS coronavirus nsp14-nsp10 complex. PNAS, USA. (2015), 112(30):9436-9441.
2. Bouvet, M. et al: RNA 3'-end mismatch excision by the severe acute respiratory syndrome coronavirus nonstructural protein nsp10/nsp14 exoribonuclease complex. Proc Natl Acad Sci USA. (2012), 109:9372-9377.
3. Subissi, L. et al: One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. Proc Natl Acad Sci USA. (2014), 111:E3900-E3909.

Catalog #

Aliquot Size

C19NS-E311H -10	10 μg
C19NS-E311H -20	20 μg
C19NS-E311H -50	50 μg

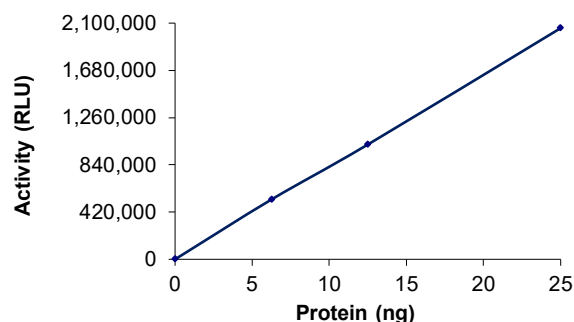
Purity



SDS-PAGE gel image

The purity of 2019-nCoV NSP14 was determined to be **>90%** by densitometry. Calculated MW **48.9 kDa**. Approx. MW **60 kDa**.

Activity



The specific activity of 2019-nCoV NSP14 Methyltransferase was determined to be **19.0 nmol/min/mg** as per the activity assay protocol.

2019-nCoV NSP14 Methyltransferase, Active

Recombinant protein expressed in *E. coli* cells

Catalog #	C19NS-E311H
Specific Activity	19.0 nmol/min/mg
Lot #	E4319-10
Purity	>90%
Concentration	0.77 $\mu\text{g}/\mu\text{l}$
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 best performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: C19NS-E311H)

Active 2019-nCoV NSP14 Methyltransferase (0.05 µg/µl) diluted with Methyltransferase Reaction Buffer II 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 NSP14 for optimal results).

Methyltransferase Reaction Buffer II

Buffer components: 50 mM Tris-HCl, pH 7.5, 3 mM MgCl₂. Add 2mM DTT (SignalChem, Catalog #: D86-09B-10) and 1 unit/µl RNasin Plus (Promega) prior to use.

MTase-Glo™ Methyltransferase Assay (Promega, Cat # V7601)

S-Adenosyl-Methionine (SAM), 1 mM
S-Adenosyl-Homocysteine (SAH), 15 µM
MTase-Glo™ Reagent, 10X
MTase-Glo™ Detection Solution, 1 bottle

Substrate

An RNA cap structure analog, G(5')ppp(5')A, was reconstituted in RNase-free water to a working stock of 5 mM.

Assay Protocol

The NSP14 assay is performed using the Methyltransferase-Glo™ Methyltransferase Assays kit (Promega).

- Step 1.** Thaw the active NSP14 and all Methyltransferase-Glo™ kit reagents on ice.
- Step 2.** Prepare the following working solutions with Methyltransferase Buffer II:
- 2.5X final concentration of Active NSP14 (Catalog #: C19NS-E311H)
 - 2.5X Substrate Cocktail: 50 µM SAM, 25 µM RNA probe
 - 5X MTase-Glo Reagent
- Step 3.** In a half-area solid white 96-well plate, add the following components to bring the initial reaction volume to 12.5 µl:
- Component 1.** 5 µl of 2.5X Substrate Cocktail
 - Component 2.** 5 µl of 2.5X Active NSP14
 - Component 3.** 2.5 µl of 5X MTase-Glo™ Reagent

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 SAH 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 plate with a plate sealer and incubate at room temperature (23°C) for 45 minutes.
- Step 5.** Add 12.5 µl of MTase-Glo™ Detection Solution to all assay wells. Mix for 2 minutes and then incubate at room temperature for 30 minutes.
- Step 6.** Read the plate using the Luminescence protocol on a GloMax® Discover Microplate Reader (Promega; Cat# GM3000).
- Step 7.** Using the SAH standard curve, determine the concentration of SAH produced (µM) and calculate the enzyme specific activity as outlined below. For a detailed protocol of how to determine SAH amount from RLUs, see MTase-Glo™ Methyltransferase Assay protocol at Promega's website: www.promega.com/protocols.

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

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

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: 2019-nCoV NSP14 Methyltransferase, Active**Catalog # C19NS-E311H**

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	1.34%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0154%
Protein	N/A	No data available	0.005%

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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