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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court West, Ste. B
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

3CL Protease (SARS-CoV) Assay Kit

Catalog #78015
Size: 96 reactions

BACKGROUND: Coronaviruses (CoVs) primarily cause multiple respiratory and intestinal infections in humans and animals. Severe acute respiratory syndrome (SARS) is a highly contagious and often fatal viral respiratory illness caused by a coronavirus called SARS-CoV. It is highly homologous to SARS-CoV-2, the causative agent for COVID-19. The 3CL protease, also known as Main Protease (Mpro), plays a vital role in processing the polyproteins that are translated from the viral RNA. 3CL Protease inhibitors that can block viral replication are promising potential drug candidates that could be used to treat patients suffering with the coronavirus infection.

DESCRIPTION: The *3CL Protease Assay Kit* is designed to measure 3CL Protease activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The kit comes in a convenient 96-well format, with purified 3CL Protease, fluorogenic substrate, and 3CL Protease assay buffer for 100 enzyme reactions. 3CL inhibitor GC376 is also included as a positive control.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100739	3CL Protease (SARS-CoV-1), MBP-tag, His-Tag	20 µg	-80°C	***Avoid freeze/ thaw cycles!***
79952	3CL Protease Substrate (5 mM)	50 µl	-80°C	
79956	3CL Protease Assay Buffer	25 ml	-20°C	
78013	GC376, MW=507.5	50 µg	-20°C	
	DTT (0.5 M)	200 µl	-20°C	
79685	Black, low binding microtiter plate with plate sealer	1	Room Temperature	

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: At least one year from date of receipt when stored as directed.

REFERENCES:

1. Morse, J.S., *et al*., 2020 *Chem.Bio.Chem*. **21:** 730 – 738.
2. Chi-Pang, C., *et al*., 2011 *PLoS ONE* **6**(11): e27228.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Add **0.5 M DTT** to **3CL Protease Assay Buffer** so final DTT concentration is 1 mM. For example, add 10 µl of **0.5 M DTT** to 5 ml assay buffer. (DTT should be added just before use. Prepare only enough DTT-containing buffer as required for the assay. Store the remaining assay buffer at -20°C).

2) Thaw **3CL Protease** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot **3CL Protease** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **3CL Protease** enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.

3) Dilute **3CL Protease** in **Assay buffer** (with 1 mM DTT) to 4 - 6.6 ng/µl (120 - 200 ng per reaction).

4) Add 30 µl **diluted 3CL Protease** enzyme solution to wells designated as "Positive Control", "Inhibitor Control" and "Test Sample". Add 30 µl **Assay buffer** (with 1 mM DTT) to the "Blank" wells.

Component	Positive Control	Test Sample	Inhibitor Control	Blank
3CL Protease (4 - 6.6 ng/µl)	30 µl	30 µl	30 µl	–
Assay Buffer (with DTT)	–	–	–	30 µl
GC376 (500 µM)	–	–	10 µl	–
Test Inhibitor	–	10 µl	–	–
Inhibitor Buffer (no inhibitor)	10 µl	–	–	10 µl
Substrate solution	10 µl	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**		**50 µl**

5) Dilute 50 µg **GC376** in 200 µl water to obtain a 500 µM solution. Add 10 µl **GC376** (500 µM) to the wells labeled "Inhibitor Control". Aliquot and store remaining solution in aliquots at -80°C.

6) Prepare the inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then

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make a 20-fold dilution in 1X assay buffer (at this step the compound concentration is 5-fold higher than the final concentration).

If the inhibitor compound is dissolved in water, make a solution of the compound 5-fold higher than the final concentration in 3CL Protease assay buffer (with 1 mM DTT). For example, diluting 50 µg GC376 in 200 µl water (step 5) creates a 500 µM solution. Adding 10 µl to the assay (final volume 50 µl) results in a 100 µM final concentration.

7) Add 10 µl inhibitor to each well designated "Test Sample". Add 10 µl 1X assay buffer or 5% DMSO (depending on which inhibitor solution is used) to "Blank" and "Positive Control" wells.
8) Preincubate enzyme with the inhibitor for 30 minutes at room temperature with slow shaking.
9) Dilute 5 mM **3CL Protease substrate** 1:20 in assay buffer with DTT, to make a 250 µM solution. Dilute only enough as is required for the assay.
10) Start reaction by adding 10 µl of the substrate solution to each well (Final concentration of the **3CL Protease substrate** in a 50 µl reaction is 50 µM).
11) Incubate at room temperature for 4-6 hours. If necessary, seal the plate with the plate sealer. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at a wavelength 360 nm and detection of emission at a wavelength 460 nm. The fluorescence intensity can also be measured kinetically. "Blank" value is subtracted from all other values.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading λexc/λem=360 nm/460 nm

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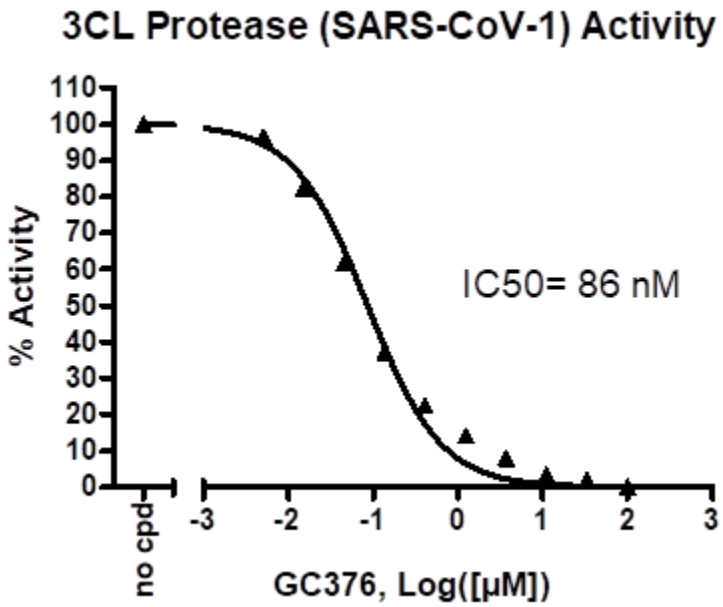
EXAMPLE OF ASSAY RESULTS:



Inhibition of 3CL Protease enzyme activity by GC376, measured using the *Fluorogenic 3CL Protease Assay Kit (BPS Bioscience #78015).* Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS

Product	Cat. #	Size
3CL Protease (SARS-CoV), MBP-tag, His-Tag	100739-1	100 µg
3CL Protease(SARS-CoV-2), MBP-tag	100707-1	100 µg
3CL Protease (SARS-CoV-2) Assay Kit	79955-1	96 reactions
PLPro, His-tag (SARS-CoV-2)	100735	20 µg/50 µg
PLPro, His-tag (SARS-CoV)	81091	25 µg
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Kit	79931	96 reactions
ACE2:SARS-CoV-2 Spike Inhibitor Screening Kit	79936	96 reactions
ACE2:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Kit	79945	96 reactions
SARS-CoV-2 Spike S1-Biotin:ACE2 TR-FRET Kit	79949	96 reactions
Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1, Fc fusion, Avi-tag, Biotin-Labeled	100679	25 µg/50 µg
Spike S1 RBD, His-tag (SARS-CoV-2)	100687	50 µg/100 µg
Spike S1, Fc fusion (SARS-CoV-2)	100688	20 µg/50 µg
Spike S1 RBD, Fc fusion (SARS-CoV-2)	100699	50 µg/100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665	20 µg/50 µg
ACE2, Fc Fusion (Monkey)	100701	50 µg/1 mg
ACE2, His-tag (Monkey)	100702	50 µg/1 mg