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Data Sheet

3CL Protease, Untagged (SARS-CoV-2) Assay Kit

Catalog #78042-2
Size: 384 reactions

Datasheet updated March 2023

BACKGROUND: Coronaviruses (CoVs) cause respiratory and intestinal infections in humans and animals. The 3CL protease, also known as Main Protease (Mpro), plays a vital role in processing the polyproteins that are translated from the viral RNA. Protease inhibitors that can block viral replication are promising potential drug candidates for the treatment of patients suffering from COVID-19 infection.

DESCRIPTION: The *Untagged 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 384-well format, with purified untagged 3CL Protease (BPS Bioscience, #100823), fluorogenic substrate, and 3CL Protease assay buffer for 400 enzyme reactions. 3CL inhibitor GC376 is also included as a positive control.

The 3CL Protease Substrate is an internally quenched 14-mer fluorogenic (FRET) peptide (DABCYL-KTSAVLQSGFRKME-EDANS). When the donor (EDANS) and acceptor (DABCYL) fluorophores are in close proximity, the energy emitted from EDANS is quenched by DABCYL (intact substrate). Upon proteolysis by 3CL, the peptide substrate is cleaved between glutamine and serine by the 3CL protease to generate the highly fluorescent peptide fragment (SGFRKME-EDANS). EDANS has an excitation peak at 336 nm and an emission peak at 455 nm. The fluorescence intensity increases proportionally to the activity of 3CL. More information on the substrate, including MW and structure, can be found on our website (BPS Bioscience, #79952).

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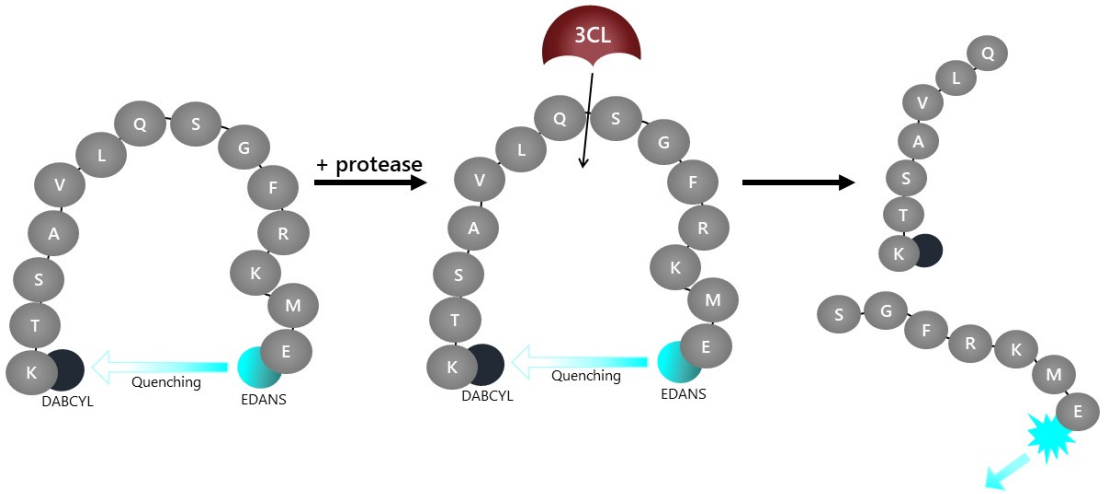

Figure 1: Illustration of the principle behind the 3CL protease assay. The 3CL Protease Substrate is an internally quenched 14-mer fluorogenic peptide (DABCYL-KTSAVLQSGFRKME-EDANS). When the donor (EDANS) and acceptor (DABCYL) fluorophores are in close proximity the energy emitted from EDANS is quenched by DABCYL (intact substrate). Upon proteolysis by 3CL, the peptide substrate is cleaved between the glutamine and serine residues to generate the highly fluorescent peptide fragment (SGFRKME-EDANS). The fluorescence intensity increases proportionally to the activity of 3CL More information on the substrate, including MW and structure, can be found on our website (BPS Bioscience #79952).

COMPONENTS:

Catalog #	Component	Amount	Storage	
100823	3CL Protease (SARS-CoV-2), no tag	6 µg	-80°C	***Avoid freeze/ thaw cycles!***
79952-1	3CL Protease Substrate (10 mM)	50 µl	-80°C	
79956	3CL Protease Assay Buffer	25 ml	-20°C	
78013	GC376, MW=507.5	2 x 50 µg	20°C	
	0.5 M DTT	200 µl	-20°C	
79961	Black, 384-well plate	1	Room Temperature	
	Plate sealing film	1		

**The exact concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

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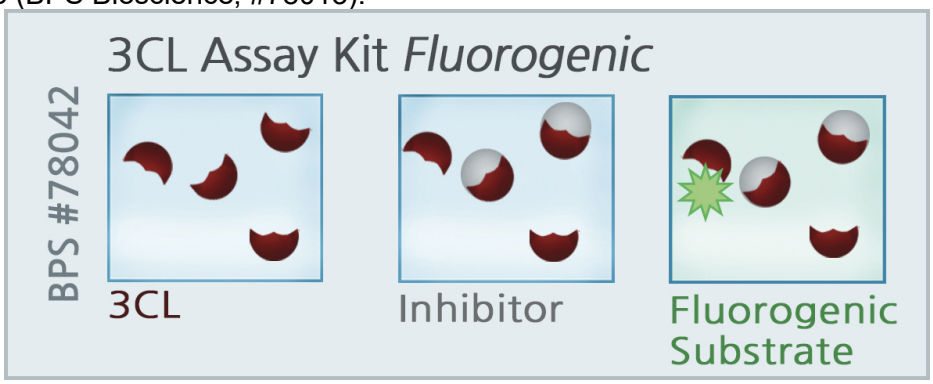
3CL inhibitor GC376 is provided as a technical control for 3CL inhibition. More information on GC376, including MW and molecular structure, can be found on our website (BPS Bioscience, #78013).



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

APPLICATIONS: Great for studying enzyme kinetics and for high throughput (HTS) applications.

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

REFERENCE(S):

1. Morse, J.S., *et al*., 2020. *Chem.Bio.Chem*. **21:**730 – 738.
2. Zhang, L., *et al.* 2020. *Science* **368 (6489):** 409-412.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Just before use, dilute **0.5 M DTT** 500 times into the **3CL Protease Assay Buffer** to obtain a DTT concentration of 1 mM. For example, add 10 µl of **0.5 M DTT** to 5 ml of assay buffer. Prepare enough DTT-containing buffer as required for the assay. Store the remaining assay buffer at -20°C.
2) Thaw the **3CL Protease** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Note: **3CL Protease** enzyme is sensitive to freeze/thaw cycles. Do not re-use the diluted enzyme.
3) Dilute **3CL Protease** in **Assay buffer** (containing 1 mM DTT) at 1.5 ng/µl (15 ng per reaction).

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Note: The exact concentration and volume of enzyme is lot-specific and will be indicated on the tube. Calculate required dilution from the information in the tube. It may be desirable to dilute the enzyme serially to avoid using large amounts of protease assay buffer for the dilution

4) Add 10 µl of **diluted 3CL Protease** to the wells designated as "Positive Control", "Inhibitor Control" and "Test Inhibitor". Add 10 µl of **Assay buffer** (containing 1 mM DTT) to the "Blank" wells.

5) Dilute the 50 µg **GC376** in 100 µl of Assay Buffer to obtain a 1000 µM solution. Add 2.5 µl of **GC376** (1000 µM) to the wells labeled "Inhibitor Control". Aliquot and store remaining solution in aliquots at -80°C

6) Prepare the Test Inhibitor.

   The final concentration of DMSO in the assay should not exceed 1%. If the Test Inhibitor is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest desired concentration. Then make a 20-fold dilution in 1x **Assay Buffer** (containing DTT). At this step the compound concentration is 5-fold higher than the final concentration.

   If the test inhibitor is dissolved in water, make a 5-fold higher concentration of the test compound than the final desired concentration in the **Assay buffer** (containing DTT).

   Add 2.5 µl of Test Inhibitor to each well designated "Test Inhibitor".

7) Diluent Solution (no inhibitor): add 2.5 µl of assay buffer (if the test compound is water soluble and was diluted in assay buffer) or 2.5 µl of 5% DMSO diluted in assay buffer (if DMSO was used to dissolve the test inhibitor) to "Blank" and "Positive Control" wells.

Component	Positive Control	Test Sample	Inhibitor Control	Blank
3CL Protease (1.5 ng/µl)	10 µl	10 µl	10 µl	–
Assay Buffer (with DTT)	–	–	–	10 µl
GC376 (1,000 µM)	–	–	2.5 µl	–
Test Inhibitor	–	2.5 µl	–	–
Diluent Solution	2.5 µl	–	–	2.5 µl
Total	**12.5 µl**	**12.5 µl**	**12.5 µl**	**12.5 µl**

8) Preincubate for 30 min at room temperature with slow shaking.

9) Dilute the 25 µl of **3CL Protease substrate** (10mM) in 3.12 ml of the **Assay Buffer** containing DTT, to make an 80 µM solution.

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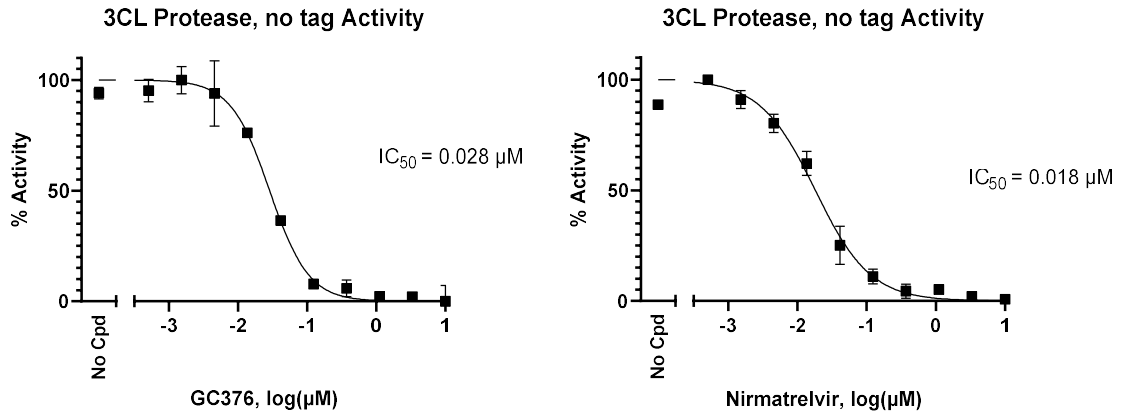
10) Start the reaction by adding 12.5 µl of the substrate solution to all the wells. The final concentration of the **3CL Protease substrate** in a 25 µl reaction is 40 µM.

    Seal the plate with the plate sealer. Incubate for 4 hours at room temperature with slow shaking.

11) Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at wavelength 360 nm and detection of emission at wavelength 460 nm. The fluorescence intensity can also be measured kinetically. "Blank" value is subtracted from all other values.

EXAMPLE OF ASSAY RESULTS:



Inhibition of 3CL Protease, untagged enzyme activity by increasing concentrations of GC376 (left) or Nirmatrelvir (right), measured using the *Untagged 3CL Protease (SARS-CoV-2) Assay Kit (BPS Bioscience, #78042-2).* Fluorescence intensity was measured using a Tecan fluorescent microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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RELATED PRODUCTS

Product	Cat. #	Size
Untagged 3CL Protease	100823-1	50 µg
Recombinant 3CL Protease, MBP-tag	100707-1	100 µg
3CL Protease (SARS-CoV-1 / SARS-CoV-2) Substrate	79952	1 mg
3CL Protease Assay Buffer	79956	25 ml
GC376	78013	50 µg
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

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