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Description

The 3CL Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2) Assay Kit is designed to measure the activity of P132H mutated, Omicron variant 3CL Protease 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 3CL Protease P132H (BPS Bioscience, #101328), fluorogenic substrate, and 3CL Protease assay buffer for 384 enzyme reactions. 3CL inhibitor GC376 is also included as a 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 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).

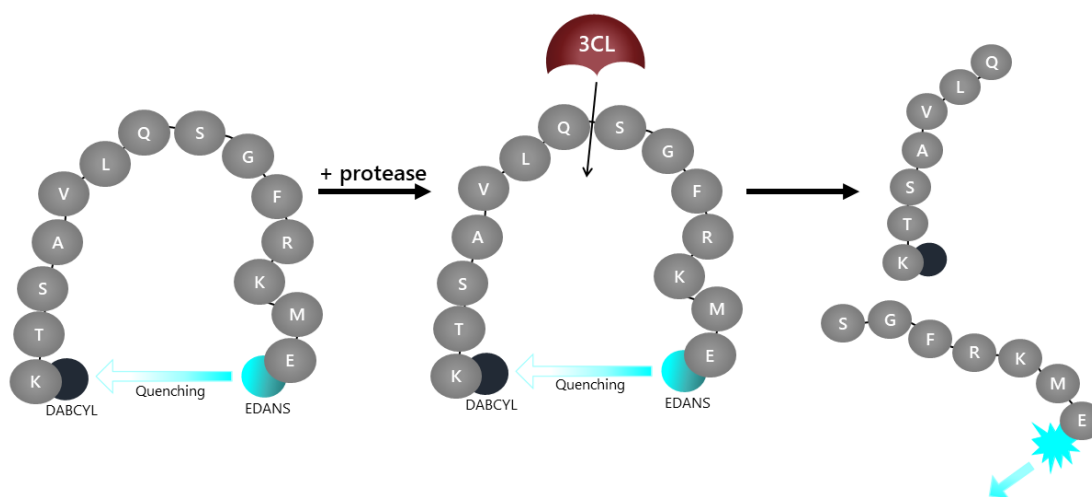


Figure: Illustration of the principle behind FRET-based 3CL protease assay

Fluorescence emitted by donor EDANS is quenched due to the proximity of the DABCYL acceptor in the intact peptide. The 3CL protease cleaves the 14-mer peptide between Q (glutamine) and S (serine), to generate the highly fluorescent peptide fragment SGFRKME-EDANS.

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.

A variant called B.1.1.529 (also known as the Omicron Variant) was identified in South Africa in November of 2021. This variant has a large number of mutations that allow the virus to spread more easily and quickly than other variants. The 3CL protease of the Omicron variant is mutated at P132H compared to the wild-type SARS-CoV-2 strain.

Mutations

P132H

Applications

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and High Throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
101328	3CL Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2)*	15 µg	-80°C
79952	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
79685	Black, 384-well plate	1	Room Temp.
	Plate sealing film	1	

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

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

Materials Required but Not Supplied

Name	Catalog #
Fluorescent microplate reader capable of reading exc/em=360 nm/460 nm	

Stability



This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety



This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

- 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.
- Thaw the **3CL Protease** (B.1.1.529, Omicron Variant) (SARS-CoV-2) on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Note: the **3CL Protease enzyme** is sensitive to

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freeze/thaw cycles. Do not re-use the diluted enzyme.

3. Dilute the **3CL Protease** (B.1.1.529, Omicron Variant) (SARS-CoV-2) in 3CL Protease Assay Buffer (containing 1 mM DTT) to 3 ng/μl (30 ng per reaction).

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** (B.1.1.529, Omicron Variant) (SARS-CoV-2) to the wells designated as “Positive Control,” “Inhibitor Control,” and “Test Inhibitor.” Add 30 μl of 3CL Protease 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 (500 μM) to the wells labeled “Inhibitor Control.” Aliquot and store remaining solution at -80°C.
6. Prepare Test Inhibitor.

Note: 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).

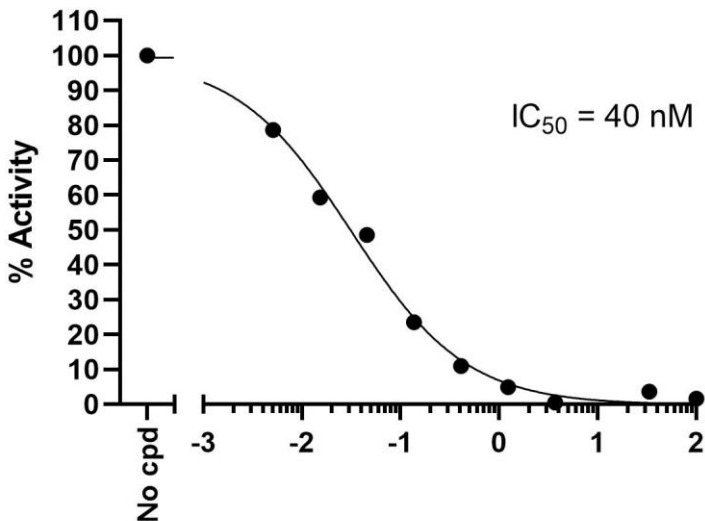
7. Add 2.5 μl of Test Inhibitor to each well designated “Test Inhibitor.”
8. Diluent Solution (no inhibitor): add 2.5 μl of assay buffer (if the test compound is water soluble and was diluted in assay buffer) or 2.5 μl of 10% DMSO diluted in assay buffer (if DMSO was used to dissolve the test inhibitor) to “Blank” and “Positive Control” wells.

Component	Blank	Positive Control	Test Inhibitor	Inhibitor Control
3CL-Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2) (3 ng/μl)	-	10 μl	10 μl	10 μl
3CL Protease Assay Buffer (with DTT)	10 μl	-	-	-
GC376 (1000 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

9. Preincubate for 30 minutes at room temperature with slow shaking.
10. Dilute 25 μl of **3CL Protease substrate** (10 mM) in 3.12 ml of the Assay Buffer containing DTT, to make an 80 μM solution. The final concentration of the 3CL Protease substrate in a 25 μl reaction is 40 μM.
11. Start the reaction by adding 12.5 μl of the substrate solution to all the wells. Seal the plate with the plate sealer and incubate for 4 hours at room temperature with slow shaking.
12. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at 360 nm and detection of emission at 460 nm. The fluorescence intensity can also be measured kinetically. “Blank” value is subtracted from all other values

Example of Assay Results

B.1.1.529, Omicron Variant Activity



GC376, Log([μM])

Inhibition of 3CL Protease enzyme activity by increasing concentrations of GC376 (BPS Bioscience, #78013), measured using the 3CL Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2) Assay Kit (BPS Bioscience, #78350-2). 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

General considerations

“Blank” Control: The “Blank” control is important to determine the background absorbance in the assay.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

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.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
3CL Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2)	101328	100 µg/1 mg
3CL Protease, Untagged (SARS-CoV-2) Assay Kit	78042	96 rxns/384 rxns
3CL Protease, MBP-tagged (SARS-CoV-2) Assay Kit	79955	96 rxns/384 rxns
3CL Protease (SARS-CoV-2)	100823	50 µg/500 µg
3CL Protease (SARS-CoV-1) Assay Kit	78015	96 rxns
3CL Protease (MERS-CoV) Inhibitor Screening Assay Kit	78278	96 rxns
3CL Protease (MERS-CoV) Assay Buffer	78022	25 ml
3CL Protease (Mpro), MBP-tag (SARS-CoV-2)	100707	100 µg/1 mg
GC376	78013	50 µg