Description

The 3CL Protease (MERS-CoV) Inhibitor Screening 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 (MERS-CoV), fluorogenic substrate, and 3CL MERS Protease assay buffer for 100 enzyme reactions. A protease inhibitor cocktail is included as a positive control.

Background

Middle East Respiratory Syndrome coronavirus (MERS-CoV) poses a significant threat to public health worldwide due to its ability to cause serious human disease with high mortality rates. MERS-CoV has become a global threat due to continuous outbreaks in countries on the Arabian Peninsula and the potential for spread to other countries. MERS-CoV 3CL protease is essential for viral replication; consequently, it is an attractive target for small molecule therapeutics.

Applications

Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100757	3CL Protease, MBP-Tag, His-tag (MERS-CoV)*	300 µg	-80°C
78021	3CL MERS-CoV substrate (1 mM)	20 µl	-80°C
78022-M	3CL MERS-CoV buffer	25 ml	-20°C
	Protease Inhibitor cocktail (10 mM in DMSO)	20 µl	-80°C
79685	Black 96-well plate	1	Room Temp.

The initial concentration of 3CL Protease (MERS-CoV) is lot-specific and will be indicated on the tube containing the protein.

Materials Required but Not Supplied

Adjustable micropipettor and sterile tips
Fluorescent microplate reader
Rotating or rocker platform

Storage Conditions

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 Principle

3CL Protease (MERS-CoV) Assay Kit uses a custom-synthesized peptide which has a fluorescence group quenched by a specific quencher. This peptide works as a substrate for 3CL Protease (MERS-CoV). After proteolytic cleavage, fluorescence product is formed and detected at λexcitation=485 nm and λemission=528 nm.

Contraindications

The final concentration of DMSO in the reaction should be ≤1%.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with the inhibitor.

Preparing Your Reagents

1. Thaw 3CL MERS assay buffer on ice.
2. Thaw 3CL Protease (MERS-CoV) on ice. Briefly spin the tube containing the enzyme to recover full contents of the tube. Calculate the amount of enzyme required for the assay and dilute enzyme to 43 ng/µl with 3CL MERS assay buffer (3000 ng/well). Aliquot the remaining undiluted 3CL protease (MERS-CoV) into single-use aliquots and store at -80°C.
   Note: 3CL Protease (MERS-CoV) is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
3. Add 70 µl of diluted 3CL Protease (MERS-CoV) to the wells designated “Positive Control” and "Test Sample" and add 70 µl of 3CL MERS assay buffer to the wells labeled as “Blank”.
4. Dilute Protease inhibitor cocktail 10-fold with 3CL MERS buffer. Prepare only the amount required for the experiment. Add 10 µl of Protease inhibitor cocktail (1 mM) to the wells labeled “Inhibitor Control”. Store the remaining undiluted solution in aliquots at -80°C.
5. Prepare the test compound by making a 100x solution. Dilute 1:10 with 3CL MERS assay buffer to make a 10x solution. Note that if the compound is dissolved in 100% DMSO, this brings the DMSO concentration to 10%. Add 10 µl of the diluted test compound to each well labeled as “Test Sample”. For the wells labeled “Positive Control" and “Blank”, add 10 µl of the diluent solution (10% DMSO in 3CL MERS assay buffer without the test compound, if diluent is DMSO).

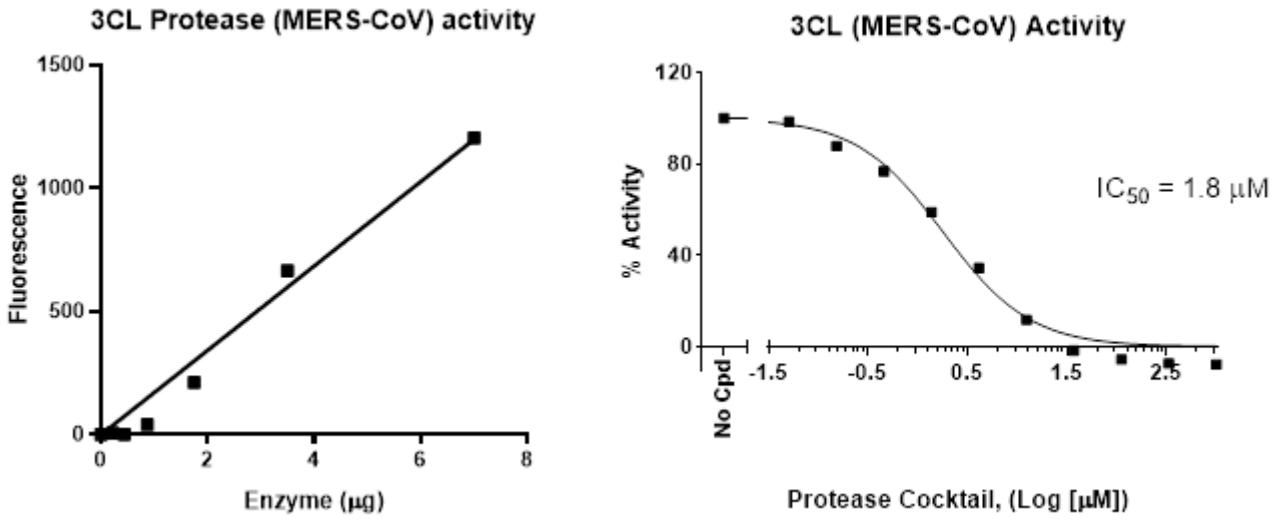
Component	Blank	Positive Control	Inhibitor Control	Test Sample
3CL Protease (MERS-CoV)	-	70 µl	70 µl	70 µl
Assay buffer	70 µl	-	-	-
Protease inhibitor cocktail	-	-	10 µl	-
Test Inhibitor	-	-	-	10 µl
Inhibitor buffer (10% DMSO in Assay buffer)	10 µl	10 µl	-	-
Total	80 µl	80 µl	80 µl	80 µl

6. Cover the plate and incubate 30 minutes at room temperature with slow shaking.
7. During the incubation, dilute 3CL MERS-CoV substrate 100-fold with Assay buffer. Dilute only the amount required for the assay. Store the remaining undiluted substrate at -80°C in single use aliquots. Discard any unused diluted substrate after use.

Initiating the reaction

8. After the 30-minute incubation, add 20 µl of diluted 3CL MERS-CoV substrate to every well. This brings the final volume to 100 µl.
9. Incubate plate at room temperature.
10. We recommend measuring the plate in a kinetic mode using a fluorimeter capable of excitation at 485±20 nm and detection of emitted light at 528±20 nm. Typically, linearity is maintained for up to 30-40 minutes. As an alternative, use end-point measurement after 30 minutes of incubation. The "Blank" value is subtracted from all other values.

Example Results



3CL Protease (MERS-CoV) activity using increasing amounts of enzyme (left) and inhibition of 3CL Protease (MERS-CoV) by increasing concentrations of Protease inhibitor cocktail (right), measured using the 3CL Protease (MERS-CoV) Inhibitor Screening Assay Kit, BPS Bioscience #78278. Fluorescence was measured using a Tecan 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 luminescence in the assay. We recommend doing these in duplicate.

"Positive Control":

The "Positive Control" is the maximum signal determined upon the addition of diluent solution (for example 1% DMSO in Assay Buffer) in the absence of inhibitor.

Troubleshooting Guide

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

Reference

Kankanamalage A., *et al.,* Eur. J. Med. Chem. 2018, **150:** 334-346.

Related Products

Products	*Catalog #*	*Size*
3CL Protease, MBP-tag, His-tag (MERS-CoV)	100757-1	100 µg
3CL Protease, MBP-tag, His-tag (MERS-CoV)	100757-2	1 mg
3CL Protease (MERS-CoV) Substrate	78021-1	50 µl
3CL Protease (MERS-CoV) Substrate	78021-2	1 mg
3CL Protease (MERS-CoV) Assay Buffer	78022	25 ml