Aurora Biolabs
High Purity Protein for Research

SARS-CoV-2 Mpro Assay Kit

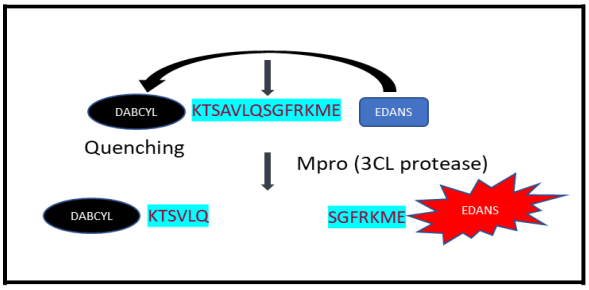
Catalog Number: 728203

Background

Mpro of Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2 Mpro, also referred to as SARS-CoV-2 Main protease, SARS-CoV-2 3CL protease) plays an essential role in viral replication by processing the polyproteins 1ab at 11 cleavage sites. Inhibiting the activity of this enzyme would block viral replication, making it a promising target for anti-coronaviral therapeutic agents.

Description

The Aurora SARS-CoV-2 Mpro assay kit is a homogeneous FRET-based assay for screening Mpro inhibitors.



The assay is fast and convenient, and requires just two steps. In the first step, the Mpro enzyme is preincubated with the compound for 30 minutes. The reaction is initiated by adding substrate solution at the second step. Fluorescent intensity is measured with a fluorescent plate reader at the excitation wavelengths of 340-360 nm and emission wavelengths of 460-480 nm.

Materials supplied

Catalogue Number	Item	Amount	Storage
728205	2X Protease Assay Buffer	20 ml	-20°C
	0.5 M DTT	200 µl	-20°C
728202	5 mM Substrate	10 µl	-80°C
728206	Recombinant SARS-CoV-2 Mpro	5 µg	-80°C
	Black low binding 96 well plate	1	RT

Materials Needed but not supplied

A microplate reader capable of measuring fluorescence at excitation wavelengths of 340-360 nm and emission wavelengths of 460-480 nm.

Stability

12 months if stored under the indicated conditions.

Assay Protocol

1. Prepare 1X assay buffer containing 1 mM DTT.

   For example, mix 996 µl distilled water with 1000 µl of 2X Protease Assay Buffer (catalogue number 728205) and 4 μl of 0.5 M DTT. Make only enough DTT-containing 1X assay buffer as needed for the assay. Store the remaining 2X Protease Assay Buffer at -20°C.

2. Prepare the inhibitor compound solution.

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 5 µl to the 50 µl reaction).

   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 make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%).

   To determine an IC_{50} or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

3. Prepare Mpro solution.

   Thaw Mpro enzyme on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.

   Note: Mpro enzyme is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted enzyme.

   Dilute the Mpro enzyme to 5 ng/µl in 1X assay buffer.

   Add 20 µl of diluted enzyme solution to each of positive control well and inhibitor test well. Add 1X buffer to each of background well.

4. Add the inhibitor solution

   Add 5 µl of 1X assay buffer to each background well and positive control well if the inhibitor is diluted in 1X buffer.

   Add 5 µl of 1X assay buffer with 10% DMSO to each of background well and positive control well if the inhibitor is diluted in 1X assay buffer with 10% DMSO.

   Add 5 µl of diluted inhibitor solution from Step 2 to each of the inhibitor test well.

5. Incubate at room temperature for 30 minutes.

6. Prepare substrate solution

   During the incubation of the enzyme and the inhibitor solution, dilute the 5 mM substrate solution to 20 µM in 1X assay buffer. Make only enough solution as need for the assay. Store the remaining 5 mM Substrate solution to -80°C.

   Add 25 µl of diluted substrate solution to each of well, including background wells, positive control wells and the inhibitor test wells.

7. Incubate at room temperature for 2 hours.

8. Measure the fluorescent intensity

   Measure the fluorescent intensity at the excitation wavelengths of 340-360 nm and the emission wavelengths of 460-480 nm

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Protocol Summary			
Component	Background	Positive Control	Inhibitor Test
1X protease assay buffer	20 µl		
Mpro enzyme (5 ng/µl)		20 µl	20 µl
Inhibitor buffer*	5 µl	5 µl	
Inhibitor solution			5 µl
	25 µl	25 µl	25 µl
Incubate at room temperature for 30 minutes.			
Substrate solution (20 µM)	25 µl	25 µl	25 µl
Final Volume	50 µl	50 µl	50 µl
Incubate at room temperature for 2 hours.			

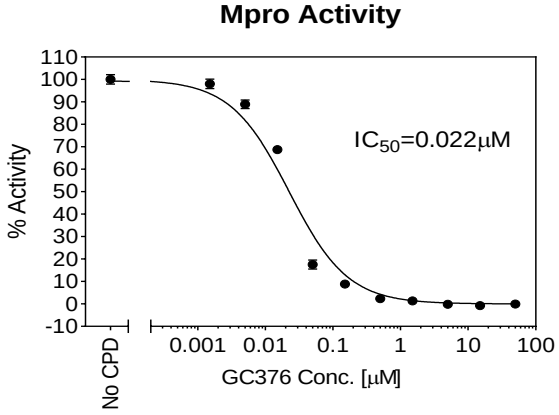
* Inhibitor buffer=1X assay buffer with 1 mM DTT if the compound is dissolved in this buffer.
* Inhibitor buffer=10% DMSO in 1X assay buffer with 1 mM DTT if the compound is dissolved in this buffer.

Data Analysis

Calculate percentage activity of the enzyme

$$\% \text{ Activity} = \frac{(Fp - Fb) - (Fi - Fb)}{Fp - Fb} \times 100$$

Where Fp refers to Fluorescent intensity of the positive control, Fb refers to Fluorescent intensity of background, and Fi refers to Fluorescent intensity of the inhibitor.
Graph the percentage activity as a function of the inhibitor concentration to determine the IC_{50} of the test inhibitor. The figure below shows the inhibitory effect of Mpro inhibitor GC376 measured using this assay kit.



No CPD refers to no compound control (compound vehicle control).

This product is for research use only and not for diagnostic or therapeutic use.

RELATED PRODUCTS:

Name of Products	Catalog#	Size
Recombinant SARS-CoV-2 Mpro, 3CL protease, CF	728201-1	50 µg
	728201-2	500ug
SARS-CoV Protease Assay Buffer (2X),	728205	20 ml
Mpro substrate, SARS-CoV-1/SARS-CoV-2	728208	1 mg
Recombinant SARS-CoV-2 Helicase (NSP13)	728231-1	10 µg
	728231-2	50 ug
	728231-3	100 ug
Recombinant SARS-CoV-2 Nucleocapsid phosphoprotein (N)	728241	100 µg
Recombinant SARS-CoV-2 Papain-like Protease (PLpro, NSP3), CF	728251-1	50 µg
	728251-2	100 µg
	728251-3	1 mg
Papain-like Protease Assay Kit	728253	96 rxns
Papain-like Protease substrate	728255	1 mg
RNA-dependent RNA polymerase (NSP12), Recombinant SARS-CoV-2	728261	100 µg
RNA-dependent RNA polymerase (NSP7, NSP8, NSP12 Complex), Recombinant SARS-CoV-2	728263	20 µg
SARS-CoV-2 NSP7, Recombinant	728264-1	100 µg
	728264-2	1 mg
SARS-CoV-2 NSP8, Recombinant	728265-1	100 µg
	728265-2	1 mg