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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Papain-like Protease (SARS-CoV-2) Assay Kit: Deubiquitinase Activity

Catalog #79996
Size: 96 reactions

BACKGROUND: Coronaviruses (CoVs) primarily cause multiple respiratory and intestinal infections in humans and animals. Papain-Like Protease (PLPro), also known as PLP, play an essential role in polypeptide processing during virus replication. PLPro, in addition to being crucial during replication via processing of the viral polyprotein, is proposed to be a key enzyme in the sustained pathogenesis of SARS-CoV. This includes deubiquitination (the removal of ubiquitin) from host-cell proteins. The last enzymatic activity result in the antagonism of the host antiviral innate immune response. As a result, PLpro is an important potential target for antiviral drugs that may inhibit viral replication and weaken dysregulation of signalling cascades in infected cells that may lead to cell death in surrounding, uninfected cells. PLPro inhibitors that can block viral replication are promising potential drug candidates that could be used to treat patients suffering with the COVID-19 coronavirus infection.

DESCRIPTION: The *Papain-like Protease Assay Kit: Deubiquitinase Activity* is designed to measure Papain-like Protease deubiquitinating 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 Papain-like Protease, ubiquitinated fluorogenic substrate, and PLPro assay buffer for 100 enzyme reactions. PLPro inhibitor GRL0617 is also included as a positive control.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100735	Recombinant Papain-like Protease, PLPro	5 µg	-80°C	***Avoid freeze/ thaw cycles!***
	25 mM PLPro Ubiquitinated Substrate	50 µl	-80°C	
	PLPro Assay Buffer	25 ml	-20°C	
	10 mM GRL0617	20 µl	-80°C	
	0.5 M DTT	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 six months from date of receipt when stored as directed.

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REFERENCE(S):

Ewelina Weglarz-Tomczak, *et al*., 2020. https://doi.org/10.1101/2020.05.17.100768.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Add **0.5 M DTT** to **PLPro 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 **PLPro** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot **PLPro** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **PLPro** enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.

3) Dilute **PLPro** in **PLPro Assay buffer** (with 1 mM DTT) at 0.7-1 ng/µl (21-30 ng per reaction).

4) Add 30 µl diluted **PLPro** 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
PLPro (0.7-1 ng/µl)	30 µl	30 µl	30 µl	–
Assay Buffer (with DTT)	–	–	–	30 µl
GRL0617 (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) Add 190 µl of **PLPro Assay buffer** (with 1 mM DTT) to 10 µl **GRL0617** to obtain a 500 µM solution. Store remaining solution in aliquots at -80°C. Add 10 µl **GRL0617** (500 µM) to the wells labeled "Inhibitor Control". Do not keep diluted **GRL0617** more than one day.

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 PLPro assay buffer (with 1 mM DTT).

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 min at 37°C.
9) Dilute 25 mM **PLPro Ubiquitinated substrate** 1:20 in assay buffer with DTT, to make a 1.25 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 **PLPro Ubiquitinated substrate** in a 50 µl reaction is 250 nM).
11) Incubate at 37°C for 45-60 minutes. 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 exc/em=360 nm/460 nm

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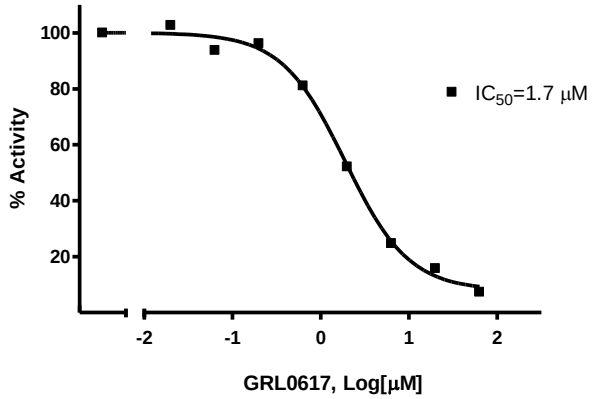
EXAMPLE OF ASSAY RESULTS:

Papain-like Protease SARS-CoV-2: Deubiquitinase Activity



Inhibition of PLPro Deubiquitination activity by GRL0617, measured using the *Papain-like Protease Assay Kit: Deubiquitinase Activity (BPS Bioscience #79996).* 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
Recombinant 3CL Protease, MBP-tag	100707-1	100 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