SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court West, Ste. B
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

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

Catalog #79995-2
Size: 384 reactions

BACKGROUND: Coronaviruses (CoVs) primarily cause multiple respiratory and intestinal infections in humans and animals. Papain-Like Protease (PLPro), also known as PLP, plays an essential role in polypeptide processing during virus replication. PLProis also proposed to be a key enzyme in the sustained pathogenesis of SARS-CoV-2. PLP acts as a deubiquitinase that removes ubiquitin and ISG15 from host-cell proteins to aid coronaviruses in their evasion of the host innate antiviral immune responses. As a result, PLPro is an important potential target for antiviral drugs that may inhibit viral replication and simultaneously weaken dysregulation of signaling 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: Protease Activity* is designed to measure Papain-like 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 Papain-like Protease, fluorogenic substrate, and PLPro assay buffer for 384 enzyme reactions. PLPro inhibitor GRL0617 is also included as a positive control.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100735	Recombinant Papain-like Protease, PLPro	2 µg	-80°C	***Avoid freeze/ thaw cycles!***
79997	PLPro Substrate (5 mM)	45 µl	-80°C	
	PLPro Assay Buffer	25 ml	-20°C	
	10 mM GRL0617	100 µl	-80°C	
	Dithiothreitol (DTT; 0.5 M)	100 µl	-20°C	
79685	Black, low binding microtiter plate	1	Room Temperature	
	Plate sealing film	2		

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: At least six months from date of receipt when stored as directed.

6042 Cornerstone Court West, Ste. B
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

REFERENCE(S):

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

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable of reading λexc/λem=360 nm/460 nm

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 0.3-0.5 ng/µl (3-5 ng per reaction).
4) Add 10 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 (0.3-0.5 ng/µl)	10 µl	10 µl	10 µl	–
Assay Buffer (with DTT)	–	–	–	10 µl
GRL0617 (1 mM)	–	–	2.5 µl	–
Test Inhibitor	–	2.5 µl	–	–
10% DMSO in water (Inhibitor buffer)	2.5 µl	–	–	2.5 µl
Substrate solution	12.5 µl	12.5 µl	12.5 µl	12.5 µl
Total	**25 µl**	**25 µl**		**25 µl**

5) Add 900 µl of **PLPro Assay buffer** (with 1 mM DTT) to 100 µl **GRL0617** to obtain a 1 mM solution. Add 2.5 µl **GRL0617** (1 mM) to the wells labeled "Inhibitor Control". Do not keep buffer-containing solution more than one day.

6042 Cornerstone Court West, Ste. B
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 make a 20-fold dilution in PLPro assay buffer with 1 mM DTT (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 2.5 µl inhibitor to each well designated "Test Sample". Add 2.5 µl 1X PLPro assay buffer with 1 mM DTT or 10% DMSO in water (depending which inhibitor solution is used) to "Blank" and "Positive Control" wells.

8) *Briefly* spin down the plate. Set on a shaker at room temperature for 5 minutes.

9) Seal the plate with a plate sealer. Preincubate enzyme with the inhibitor for 60 minutes at 37°C.

10) Dilute 5 mM **PLPro substrate** 1:120 in assay buffer with DTT, to make a 42 µM solution. Dilute only enough as is required for the assay.

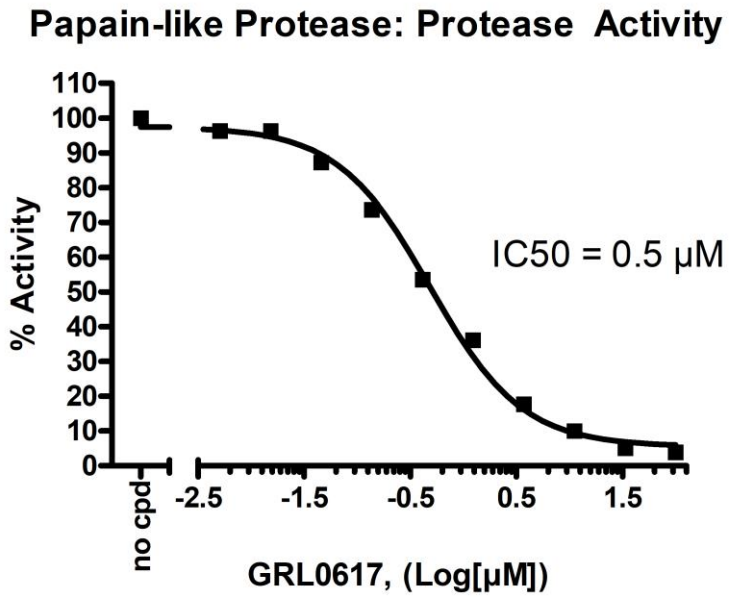
11) Start reaction by adding 12.5 µl of the substrate solution to each well (Final concentration of the **PLPro substrate** in a 25 µl reaction is 21 µM).

12) *Briefly* spin down the plate. Set on a shaker at room temperature for 5 minutes.

13) Reseal the plate with a new plate sealer and 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.

6042 Cornerstone Court West, Ste. B
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

EXAMPLE OF ASSAY RESULTS:



Inhibition of PLPro Protease enzyme activity by GRL0617, measured using the *Papain-like Protease Assay Kit: Protease Activity (BPS Bioscience #79995-2).* 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*

6042 Cornerstone Court West, Ste. B
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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