


EnzChek™ 3C-like Protease (3CLpro) and Papain-like Protease (PLpro) Assay Kits

Catalog Numbers E33970, E33971, E33972, and E33973

Pub. No. MAN0025588 Rev. A.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

The EnzChek™ 3C-like Protease (3CLpro) and Papain-like Protease (PLpro) Assay Kits are rapid, simple, and direct fluorescence-based assays for detecting SARS-CoV-2 3-chymotrypsin-like protease (3CLpro) and SARS-CoV-2 Papain-like Protease (PLpro) activity. Coronavirus 3C-like protease (3CLpro), also known as Main Protease (Mpro), and Papain-like protease (PLpro) are a crucial enzymes for processing viral polyproteins, and, because of their role in the viral life cycle, have been identified as promising drug targets for stopping viral replication and treating COVID-19 infection.

Our EnzChek™ kits are designed for screening and profiling inhibitors of 3CLpro and PLpro. Everything needed to determine protease activity is included, including a positive control inhibitor. Each 3CLpro and PLpro kit contains a protease-specific fluorogenic substrate, which, upon protease catalyzed hydrolysis, releases the fluorescently labeled peptide. Protease activity can be directly correlated to the change in signal using a microplate reader. These EnzChek™ assays are homogeneous assays that require no washing steps. The reagents supplied with each kit are sufficient to perform 100 or 5 × 100 microwell assays.

Contents and storage

Product	Amount	Cat. No.
EnzChek™ 3C-like Protease (3CLpro) Assay Kit	100 assays	E33970
	5 × 100 assays	E33971
EnzChek™ Papain-like Protease (PLpro) Assay Kit	100 assays	E33972
	5 × 100 plates	E33973

Table 1 EnzChek™ 3C-like Protease (3CLpro) Assay Kit components

Component	Amount (100 assays)	Amount (5 × 100 assays)	Storage	Stability
3C-like Protease (3CLpro) Assay Buffer	10 mL	50 mL	-20°C	<ul style="list-style-type: none"> Kit is stable for a minimum of 6 months when stored as directed. Avoid freeze/thaw cycles.
Buffer Additive A	50 µL	5 × 50 µL		
3C-like Protease (3CLpro) SARS-Cov-2 (100 µg/mL)	2 µg	5 × 2 µg	-80°C	
3C-like Protease (3CLpro) Fluorescent Substrate (10 mM in DMSO)	25 µL	5 × 25 µL		
GC376 (MW 507.5)	50 µg	50 µg		

Table 2 EnzChek™ Papain-like Protease (PLpro) Assay Kit components

Component	Amount (100 assays)	Amount (5 × 100 assays)	Storage	Stability
Papain-like Protease (PLpro) Assay Buffer	10 mL	50 mL	–20°C	<ul style="list-style-type: none"> • Kit is stable for a minimum of 6 months when stored as directed. • Avoid freeze/thaw cycles.
Buffer Additive A	50 µL	5 × 50 µL		
Papain-like Protease (PLpro) SARS-CoV-2 (100 µg/mL)	2 µg	5 × 2 µg	–80°C	
Papain-like Protease (PLpro) Fluorescent Substrate (5 mM)	30 µL	5 × 30 µL		
GRL0617 (10 mM in DMSO)	20 µg	100 µg		

Required materials not supplied

- Fluorescence microplate reader capable of reading Ex/Em 360 nm/460 nm
- Microtiter plate with plate sealer (recommend Corning™ 96-Well, Flat-Bottom Polystyrene NBS Microplate, Cat. No. [07-201-203](#))
- Inhibitor of choice

Protocols

The solution volumes recommended in this section provide sufficient reagents for a 96-well plate using a fluorescent microplate reader and 50 µL per microplate well. Stock solutions should only be diluted immediately prior to use and assay buffer with Buffer Additive A should be prepared fresh.

Detect 3C-like Protease

Prepare 3C-like Protease reagents

1. Prepare the assay buffer by adding 10 µL of Buffer Additive A to 5 mL of assay buffer. Add Buffer Additive A just prior to use. Do not store the mixed assay buffer.
2. Prepare a 200 µM solution of 3C-like Protease (3CLpro) Fluorescent Substrate by diluting 1:50 in prepared assay buffer.
Note: Mix the substrate well to fully dissolve and use immediately after preparation. Each well requires 10 µL of 200 µM substrate solution.
3. Thaw 3C-like Protease on ice and centrifuge tube to recover contents.
4. Dilute 3C-like Protease to 0.67 ng/µL using prepared assay buffer by combining 20 µL of 3C-like Protease with 2980 µL of prepared assay buffer in a separate vial (not supplied) for a total volume of 3000 µL. Each well requires 20 ng (30 µL of 0.67 ng/µL) of 3C-like Protease.
5. Dilute inhibitor of choice to desired concentration in prepared assay buffer. If the inhibitor is dissolved in DMSO, the final concentration of DMSO should not exceed 1%.
6. (Optional) GC376, a 3CL protease inhibitor, has been included as a positive control. It is recommended to create a 500 µM solution by dissolving 50 µg of GC376 in 200 µL of assay buffer. Solution may be aliquoted and stored at –80°C for future use.

Assess 3C-like Protease activity

Using a 96-well plate:

1. Add 30 μL of diluted 3C-like Protease. Use prepared assay buffer without protease for the blank.
2. Add 10 μL of inhibitor solution to the desired wells and incubate for 30 minutes at room temperature.
3. Initiate the reaction by adding 10 μL of 3C-like Protease Substrate to each well.
4. Incubate at room temperature for 1 hour.
5. Measure fluorescence at 360 nm/460 nm (Ex/Em).

Detect Papain-like Protease

Prepare Papain-like Protease

1. Prepare the assay buffer by adding 10 μL of Buffer Additive A to 5 mL of assay buffer. Add Buffer Additive A just prior to use. Do not store the mixed assay buffer.
2. Prepare 1 mL of Papain-like Protease (PLpro) Fluorescent Substrate by diluting 1:40 in prepared assay buffer to make a 125 μM solution.
Note: Mix substrate well to fully dissolve and use immediately after preparation. Each well requires 10 μL of 125 μM substrate solution.
3. Thaw Papain-like Protease (PLpro) SARS-Cov-2 on ice and centrifuge tube to recover contents.
4. Dilute Papain-like Protease (PLpro) in prepared assay buffer in a separate vial (not supplied) for a total volume of 3000 μL . Each well requires 20 ng (30 μL of 0.67 ng/ μL) of protease.
5. Dilute inhibitor of choice to desired concentration in prepared assay buffer. If the inhibitor is dissolved in DMSO, the final concentration of DMSO in the assay should not exceed 1%.
6. *(Optional)* GRL0617 (10 mM in DMSO), a Papain-like protease inhibitor, has been included as a positive control. It is recommended to create a 500 μM solution by dissolving 10 μL of GRL0617 in 190 μL of assay buffer.

Assess Papain-like Protease activity

Using a 96-well plate:

1. Add 30 μL of diluted protease. Use prepared assay buffer without protease for the blank.
2. Add 10 μL of inhibitor solution to the desired wells and incubate for 30 minutes at room temperature.
3. Initiate the reaction by adding 10 μL of Papain-like Protease substrate to each well.
4. Incubate at 37°C for 1 hour.
5. Measure fluorescence at 360 nm/460 nm (Ex/Em).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.



The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0025588

Revision	Date	Description
A.0	08 October 2021	New manual for new product launch.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2021 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.