Description

The Cathepsin L Inhibitor Screening Assay Kit is designed to measure the protease activity of Cathepsin L for screening and profiling applications. The Cathepsin L assay kit comes in a convenient 384-well format, with purified Cathepsin L (amino acids 18-333), its substrate, and Cathepsin Buffer for 384 reactions. This kit includes the inhibitor E-64 as control.

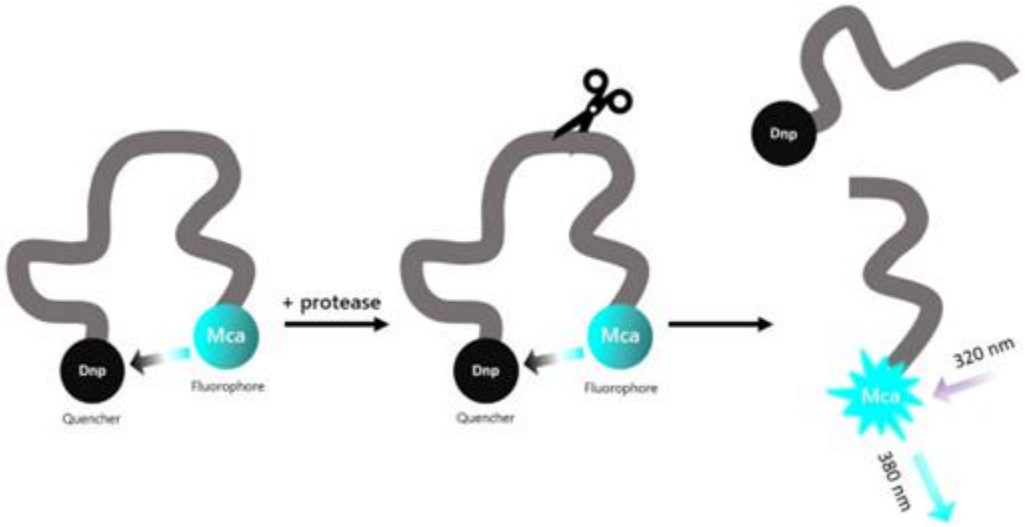

Figure 1: Illustration of the assay principle.

The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent Mca from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

Cathepsin L is a lysosomal cysteine protease that belongs to the papain-like superfamily. It is involved in many essential physiological processes, including degradation and renewal of intracellular proteins, activation of prohormones, presentation of antigens, as well as organ development. One of its main functions is in the degradation of antigens, and it has been shown to participate in SARS-CoV-1 and SARS-CoV-2 infection, as infection requires viral membrane fusion and that seems to require proteolysis of the Spike protein by Cathepsin L. The use of amantadine inhibited Cathepsin L and prevented SARS-CoV-2 infection. Cathepsin L plays important roles in tumor metastasis and chemotherapy resistance, and upregulation of this enzyme has been reported in a wide range of human cancers including ovarian, renal, and breast carcinoma. The development of inhibitors targeting this protein may prove beneficial for both COVID-19 and cancer therapy.

Applications

Screen small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80005	Cathepsin L, His-Tag*	1 µg	-80°C
80349	Fluorogenic Cathepsin Substrate 1 (5 mM)	2 x 10 µl	-20°C
	4x Cathepsin Buffer	2 x 2 ml	-20°C
	0.5 M DTT	2 x 200 µl	-20°C
	1 mM E-64	10 µl	-20°C
79961	384-well black microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Fluorescence plate reader capable of measurement at λex330/λem390 nm.

Stability

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 Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Negative Control", "Positive Control", "Control Inhibitor" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using E-64 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Add 120 µl of **0.5 M DTT** to one vial (2 ml) of **4x Cathepsin Buffer**.

2. Prepare 1x Cathepsin Buffer by diluting 4x Cathepsin Buffer 4-fold with distilled water.

3. Thaw **Cathepsin L**, on ice. Briefly spin the tube to recover the full content.

4. Dilute Cathepsin L to 0.02 ng/µl with 1x Cathepsin Buffer (10 µl/well).

5. Prepare the Test Inhibitor (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x Cathepsin Buffer.

   For the positive and negative controls, use 1x Cathepsin Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Cathepsin Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 1x Cathepsin Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x Cathepsin Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 10 µl of diluted Cathepsin L to all wells, except "Negative Control" wells.

7. Add 10 µl of 1x Cathepsin Buffer to the "Negative Control" wells.

8. Prepare the Inhibitor Control by diluting 1 mM E-64 to 1000X the IC_{50} in 100% DMSO. Then dilute 10-fold in 1x Cathepsin Buffer (the DMSO amount is now 10%) and corresponds to 100X the IC_{50} value (2.5 µl/well). Using Diluent Solution prepare solutions at 1X and 10X the IC_{50} value (2.5 µl/well).

9. Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor".

10. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

11. Add 2.5 µl of diluted E-64 to the "Control Inhibitor" wells.

12. Preincubate the "Test Inhibitor" with the diluted Cathepsin L for 30 minutes at Room Temperature (RT) with gentle agitation.

13. Dilute 500-fold the Fluorogenic Cathepsin Substrate 1 with 1x Cathepsin Buffer (12.5 µl/well).

14. Start the reaction by adding 12.5 µl of the diluted Fluorogenic Cathepsin Substrate 1 to all wells. Protect your samples from direct exposure to light.

15. Incubate at RT for 60 minutes or perform kinetic analysis.

16. Read fluorescence intensity of the samples (lexcitation = 360 nm; lemission = 460 nm) in an appropriate microplate reader.

Component	Negative Control	Positive Control	Control Inhibitor	Test Inhibitor
1x Cathepsin Buffer	10 µl	-	-	-
Test Inhibitor	-	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-	-
Diluted E-64	-	-	2.5 µl	-
Diluted Cathepsin L (0.02 ng/µl)	-	10 µl	10 µl	10 µl
30 minutes at Room Temperature				
Diluted Fluorogenic Cathepsin Substrate 1 (500-fold)	12.5 µl	12.5 µl	12.5 µl	12.5 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Example Results

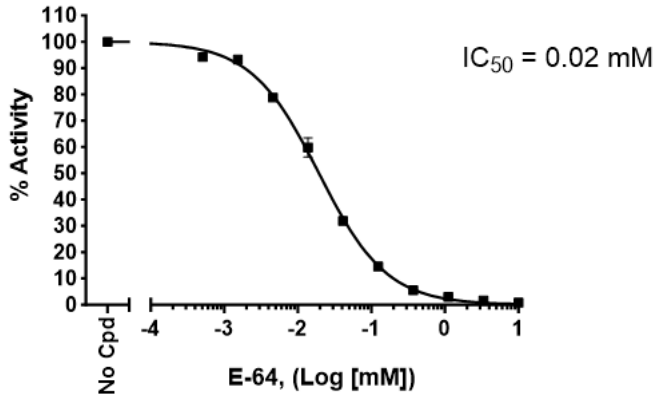

Figure 2: Inhibition of Cathepsin L activity by E-64.
Cathepsin L activity was measured in the presence of increasing concentrations of E-64. Results are expressed as percent of control (Cathepsin L activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

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

References

Zhao M., *et al.*, 2021 *Signal Transduction and Targeted Therapy* 6:134.

Related Products

Products	*Catalog #*	*Size*
Cathepsin D, His-Tag Recombinant	101391	10 µg
Cathepsin B, His-Tag Recombinant	80001	10 µg
Cathepsin D Inhibitor Screening Assay Kit	82141	96 reactions/384 reactions
Cathepsin B Inhibitor Screening Assay Kit	79590	96 reactions/384 reactions

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