

Description

The Cathepsin D Inhibitor Screening Assay Kit is designed to measure the protease activity of Cathepsin D for screening and profiling applications. The Cathepsin D assay kit comes in a convenient 96-well format, with enough recombinant human Cathepsin D (amino acids 21-412), its substrate, and Cathepsin buffer for 96 reactions. This kit contains Pepstatin A as internal control.

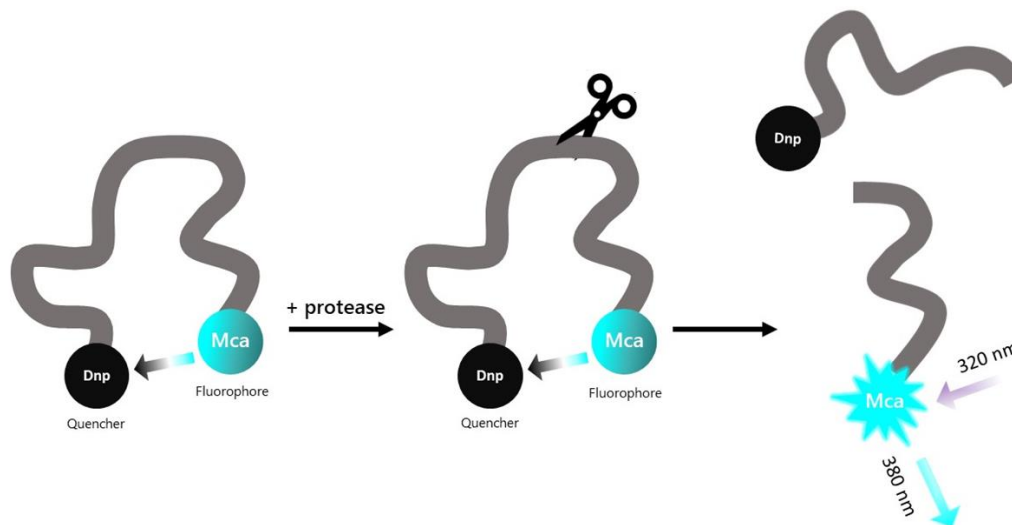


Figure 1: Illustration of the assay principle.

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 D is a lysosomal aspartyl protease, of the peptidase A1 family. It is involved in lysosomal protein degradation and activation of proteins that are synthesized as precursors, such as hormones and growth factors. Its activity impacts cell death and inflammation. Cathepsin D dysfunction has been linked to breast and gastric cancer, Alzheimer's disease (AD) and neuronal ceroid lipofuscinosis (NCL). Overexpression of this protein can lead to activation of VEGF-C (vascular endothelial growth factor -C) and VEGF-D and metastasis and angiogenesis. Cathepsin D is therefore a promising new therapeutic target. It has been shown that an antibody against Cathepsin D was able to inhibit grow of triple-negative breast cancer cells. Cathepsin D inhibition is also a promising approach in combination treatments, by maximizing the effect of other anti-cancer drugs.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
101391	Cathepsin D, His-Tag*	>1 µg	-80°C
	CS Substrate 2	12.5 µl	-80°C
	4x Cathepsin Buffer	2 ml	-20°C
	0.5 M DTT	200 µl	-80°C
	10 mM Pepstatin A	5 µl	-80°C
79685	96-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 $\lambda_{ex}330/\lambda_{em}390$ 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.
 - If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.
1. Add 120 µl of **0.5 M DTT** to **4x Cathepsin Buffer**.
 2. Prepare 1x Cathepsin Buffer by diluting 4x Cathepsin Buffer 4-fold with distilled water.
 3. Thaw **Cathepsin D**, on ice. Briefly spin the tube to recover the full content.
 4. Dilute Cathepsin D to 0.25 ng/µl with 1x Cathepsin Buffer (20 µl/well).

Note: Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

5. Prepare the Test Inhibitor (5 μ l/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 μ l.

5.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using 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 20 μ l of diluted Cathepsin D to all wells except the "Negative Control".
7. Add 20 μ l of 1x Cathepsin Buffer to the "Negative Control" wells.
8. Dilute 10 mM Pepstatin A 1000-fold with 100% DMSO to get a 10 μ M solution.
9. Dilute 10 μ M Pepstatin A 10-fold with 1x Cathepsin Buffer to get a 1 μ M solution.
10. Add 5 μ l of inhibitor solution to each well designated "Test Inhibitor".
11. Add 5 μ l of Diluent Solution to the "Positive Control" and "Negative Control" wells.
12. Add 5 μ l of diluted Pepstatin A (1 μ M) to the "Control Inhibitor" wells.
13. Preincubate the "Test Inhibitor" with the diluted Cathepsin D for 30 minutes at Room Temperature (RT) with gentle agitation.
14. Dilute 200-fold the CS Substrate 2 with 1x Cathepsin Buffer.
15. Add 25 μ l of the diluted CS Substrate 2 to all wells. Protect your samples from direct exposure to light.
16. Incubate at RT for 30-60 minutes or perform kinetic analysis.

17. Read fluorescence intensity of the samples (lexcitation = 330 nm; lemission = 390 nm) in a fluorescence microplate reader.

Component	Negative Control	Positive Control	Control Inhibitor	Test Inhibitor
1x Cathepsin Buffer	20 μ l	-	-	-
Test Inhibitor	-	-	-	5 μ l
Diluent Solution	5 μ l	5 μ l	-	-
Diluted Pepstatin A (1 μ M)	-	-	5 μ l	-
Diluted Cathepsin D (0.25 ng/ μ l)	-	20 μ l	20 μ l	20 μ l
Diluted CS Substrate 2 (diluted 200-fold)	25 μ l	25 μ l	25 μ l	25 μ l
Total	50 μl	50 μl	50 μl	50 μl

Example Results

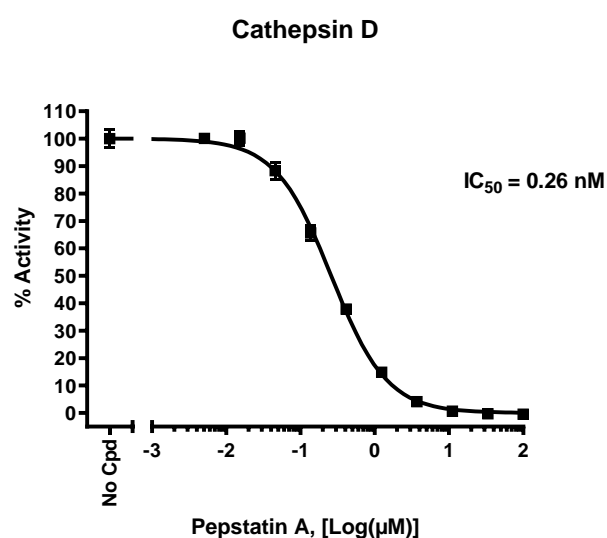


Figure 1. Inhibition of Cathepsin D activity by Pepstatin A.

Cathepsin D activity was measured in the presence of increasing concentrations of Pepstatin A. The Blank value was subtracted from all other values. Results are expressed as percent of control (Cathepsin D 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

Seo S., et al., 2022 *Cell Death and Disease* 13: 115.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Cathepsin E, His-Tag Recombinant	11070	10 µg
Cathepsin B, His-tag Recombinant	80001	10 µg
Cathepsin E Inhibitor Screening Assay Kit	82110	96 reactions/384 reactions
Cathepsin B Inhibitor Screening Assay Kit	79590	96 reactions/384 reactions