

Description

The Cathepsin F Inhibitor Screening Assay Kit is designed to measure the protease activity of Cathepsin F for screening and profiling applications. The Cathepsin F assay kit comes in a convenient 96-well format, with enough purified Cathepsin F (amino acids 20-484), its substrate, and Cathepsin Buffer for 96 reactions.

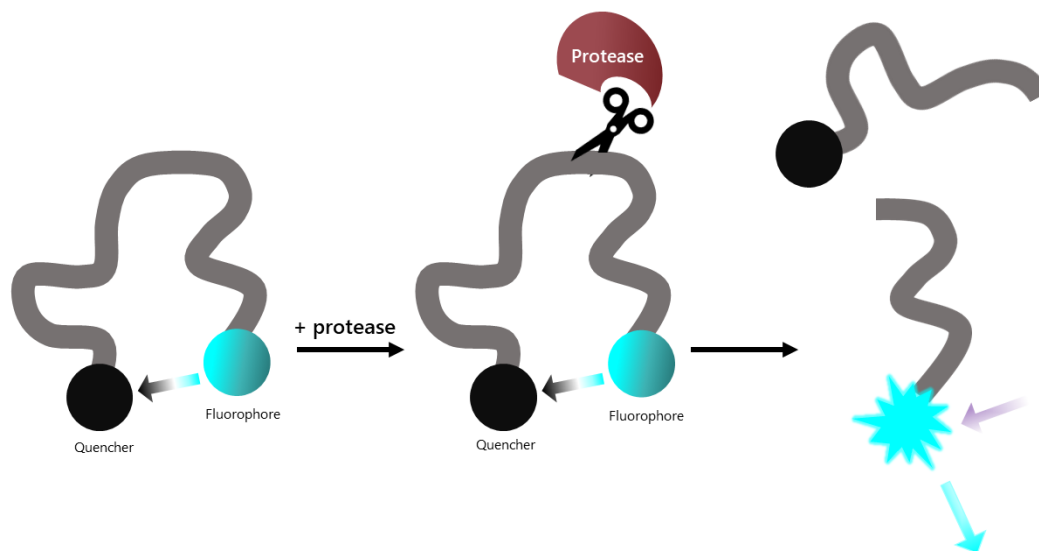


Figure 1: Illustration of the assay principle.

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

Background

Cathepsin F is a lysosomal cysteine protease that belongs to the papain-like superfamily. It is secreted by macrophages and is thought to play a role in processing and loading peptides into MHC complexes. Cathepsin F is also expressed in atherosclerotic lesions, where it degrades ApoB-100, triggering LDL (low density lipoprotein) aggregation. It has been implicated in tumor invasion and metastasis, and when it is downregulated, it can induce apoptosis of gastric cancer cells. Mutations in cathepsin F have been linked to Alzheimer's disease and Type B Kuf's disease. A deeper understanding of the mechanisms of action of cathepsin F, and development of specific inhibitors, may result in new cancer therapy avenues.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80003	Cathepsin F, His-Tag*	>1 µg	-80°C
80350	Fluorogenic Cathepsin F Substrate (0.5 mM)	100 µl	-20°C
	4x Cathepsin Buffer	2 ml	-20°C
	0.5 M DTT	200 µl	-20°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 $\lambda_{ex}360/\lambda_{em}460$ 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 Cystatin C 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 IC₅₀ value shown in the validation data below.
1. Add 120 µl of **0.5 M DTT** to 2 ml of **4x Cathepsin Buffer**.
 2. Prepare 1x Cathepsin Buffer by diluting 4x Cathepsin buffer 4-fold with distilled water.
 3. Thaw **Cathepsin F**, on ice. Briefly spin the tube to recover the full content.
 4. Dilute Cathepsin F to 10 ng/µl with 1x Cathepsin Buffer.
 5. Dilute 10 ng/µl Cathepsin F to 0.5 ng/µl with 1x Cathepsin Buffer (20 µl/well).

6. 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.

6.1 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

6.2 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%.

7. Add 20 μ l of diluted Cathepsin F to all wells, except the "Negative Control" wells.
8. Add 20 μ l of 1x Cathepsin Buffer to the "Negative Control" wells.
9. Add 5 μ l of inhibitor solution to each well designated "Test Inhibitor".
10. Add 5 μ l of Diluent Solution to the "Positive Control" and "Negative Control" wells.
11. Preincubate the inhibitors with the diluted Cathepsin F for 30 minutes at Room Temperature (RT) with gentle agitation.
12. Dilute 25-fold the Fluorogenic Cathepsin F Substrate with 1x Cathepsin Buffer to make a 20 μ M solution (25 μ l/well).
13. Start the reaction by adding 25 μ l to every well. Protect your samples from direct exposure to light.
14. Incubate at RT for 60 minutes or perform kinetic analysis.
15. Read fluorescence intensity of the samples ($\lambda_{\text{excitation}} = 360 \text{ nm}$; $\lambda_{\text{emission}} = 460 \text{ nm}$) in an appropriate microplate reader.

Component	Negative Control	Positive Control	Test Inhibitor
1x Cathepsin Buffer	20 μ l	-	-
Test Inhibitor	-	-	5 μ l
Diluent Solution	5 μ l	5 μ l	-
Diluted Cathepsin F (0.5 ng/ μ l)	-	20 μ l	20 μ l
30 minutes at Room Temperature			
Diluted Fluorogenic Cathepsin F Substrate (25-fold)	25 μ l	25 μ l	25 μ l
Total	50 μl	50 μl	50 μl

Example Results

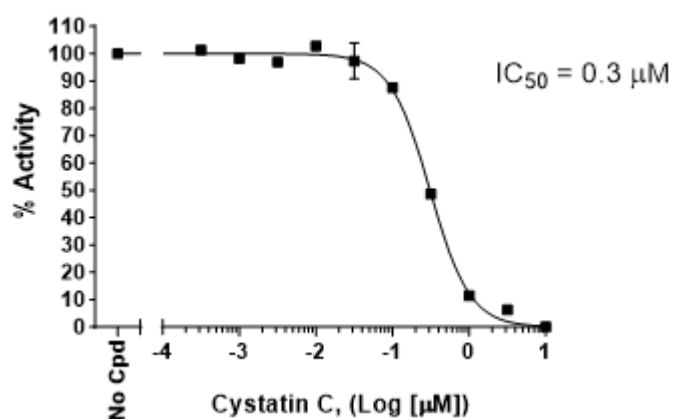


Figure 2: Inhibition of Cathepsin F activity by Cystatin C.

Cathepsin F activity was measured in the presence of increasing concentrations of Cystatin C. Results are expressed as percent of control (Cathepsin F 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

Smith K R., et al., 2013. *Human Molec. Genetics* 22(7): 1417-1423.
 Ji C., et al., 2018 *Oncol Res* 26(1): 83-93.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
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

Version 120723