Description

The Cathepsin B Inhibitor Screening Assay Kit is designed to measure the protease activity of Cathepsin B for screening and profiling applications. The Cathepsin B assay kit comes in a convenient 96-well format, with enough purified Cathepsin B (amino acids 18-339), its substrate, and Cathepsin Buffer for 96 reactions. This kit includes the inhibitor E-64 as control.

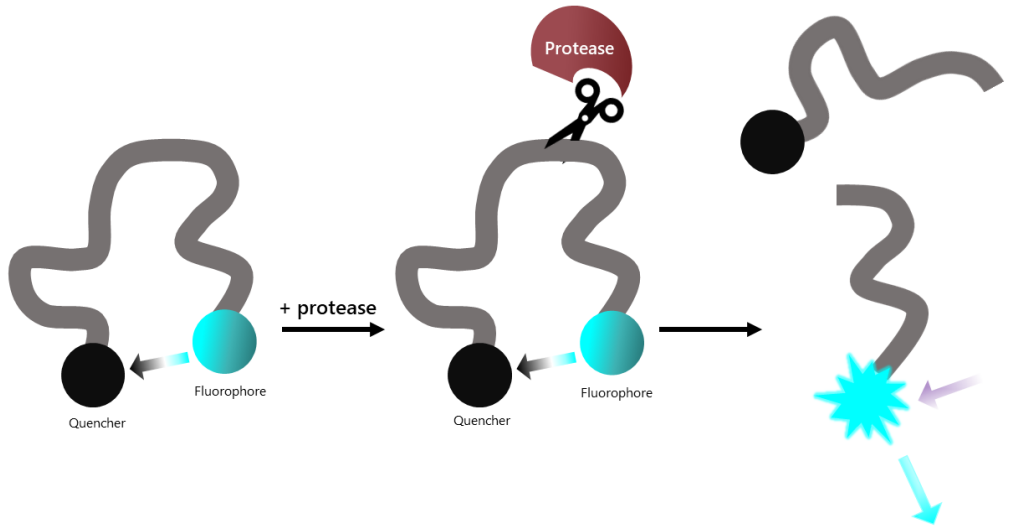

Figure 1: Illustration of the assay principle.
The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent substrate from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

Cathepsin B is a cysteine protease, of the C1 family of papain-like peptidases, that primarily functions as an endopeptidase within endo-lysosomal compartments in normal cells. Cathepsin B is closely linked to apoptosis, with activated caspase-8 leading to the release of cathepsin B to the cytosol, which in turn leads to cytochrome c release and apoptosis. It is also involved in Bcl-2 (B-cell lymphoma 2) degradation. It plays a role in inflammasome regulation, with NLRP3 (NOD and pyrin containing protein 3) activators increasing the interaction cathepsin-NLRP3 and caspase-1 activation. Cathepsin B-mediated programmed cell death (PCD) can be a cause of disease progression in abnormal tissues. High levels of cathepsin B are found in a wide variety of human cancers and in experimental models, such as transgenic models of murine pancreatic and mammary carcinomas and causal roles for this protein have been demonstrated in initiation, growth/tumor cell proliferation, angiogenesis, invasion, and metastasis. It has been proposed as a biomarker, and its levels seem to correlate with metastasis. Its multiple roles make cathepsin B an attractive target for cancer therapy.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80001	Cathepsin B, His-Tag*	1 µg	-80°C
80349	Fluorogenic Cathepsin Substrate 1 (5 mM)	10 µl	-20°C
	4x Cathepsin Buffer	2 ml	-20°C
	0.5 M DTT	200 µl	-20°C
	1 mM E-64	10 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 λex360/λem460 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 2 ml of **4x Cathepsin Buffer**.

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

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

4. Dilute Cathepsin B to 0.02 ng/µl with 1x Assay Buffer (20 µl/well).

5. Prepare the Test Inhibitor (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 5 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 20 µl of diluted Cathepsin B to all wells, except the "Negative Control" wells.

7. Add 20 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 (5 µl/well). Using Diluent Solution prepare solutions at 1X and 10X the IC_{50} value (5 µl/well).

9. Add 5 µl of inhibitor solution to each well designated "Test Inhibitor".

10. Add 5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

11. Add 5 µl of diluted E-64 to the "Control Inhibitor" wells.

12. Preincubate the Inhibitors with the diluted Cathepsin B for 30 minutes at Room Temperature (RT) with gentle agitation.

13. Dilute 500-fold the Fluorogenic Cathepsin Substrate 1 with 1x Cathepsin Buffer (25 µl/well).

14. Start the reaction by adding 25 µl of the diluted Substrate 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 (λexcitation = 360 nm; λemission = 460 nm) in an appropriate microplate reader.

Component	Negative Control	Positive Control	Control Inhibitor	Test Inhibitor
1x Cathepsin Buffer	20 µl	-	-	-
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	-	-
Diluted E-64	-	-	5 µl	-
Diluted Cathepsin B (0.04 ng/µl)	-	20 µl	20 µl	20 µl
30 minutes at Room Temperature				
Diluted Fluorogenic Cathepsin Substrate 1 (500-fold)	25 µl	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Example Results

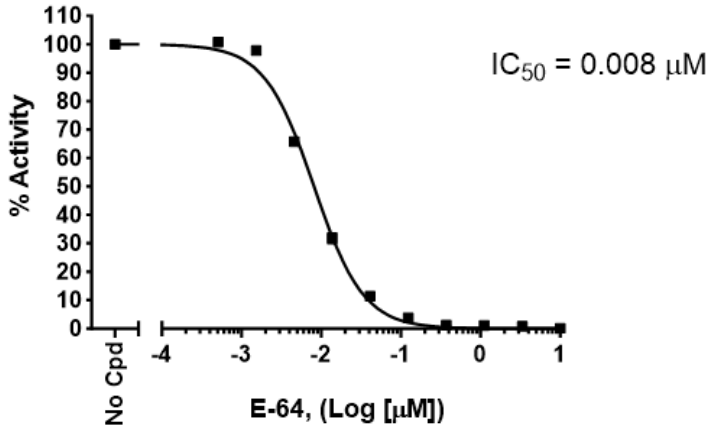

Figure 2: Inhibition of Cathepsin B activity by E-64.
Cathepsin B activity was measured in the presence of increasing concentrations of E-64. Results are expressed as percent of control (Cathepsin B activity in the absence of inhibitor, set at 100%).

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
Xie Z., *et al.*, 2023 *Cell Death & Disease* 14: 255.
Chevriaux A., *et al.*, 2020 *Front. Cell Dev. Biol*. 8: https://doi.org/10.3389/fcell.2020.00167.

Related Products

Products	*Catalog #*	*Size*
Cathepsin D, His-Tag Recombinant	101391	10 µg
Cathepsin D Inhibitor Screening Assay Kit	82141	96 reactions/384 reactions
Cathepsin B Inhibitor Screening Assay Kit	79590	96 reactions/384 reactions
NLRP3 (NALP3), His-FLAG-Tag Recombinant	100189	10 µg
NLRP3 Human shRNA Lentivirus	82122	500 µl x 2

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