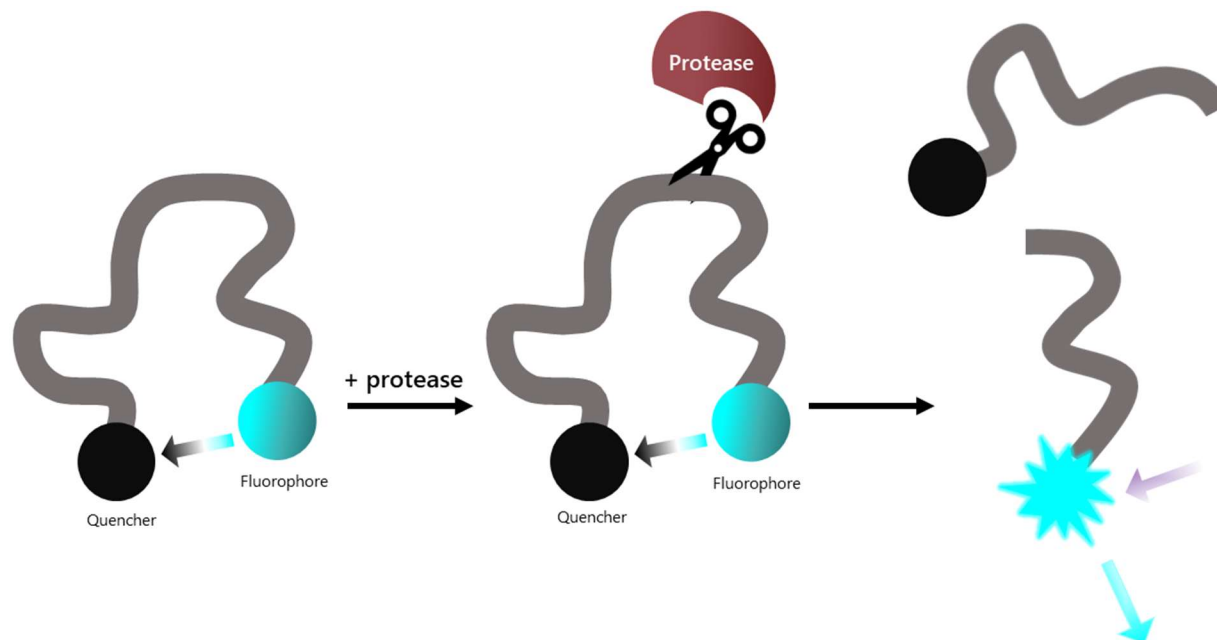


## Description

The Cathepsin E Inhibitor Screening Assay Kit is designed to measure the protease activity of Cathepsin E for screening and profiling applications. The Cathepsin E assay kit comes in a convenient 384-well format, with enough recombinant human Cathepsin E (amino acids 18-396), its substrate, and Cathepsin buffer for 384 reactions. This kit includes inhibitor Pepstatin A 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 E, also known as erythrocyte membrane aspartic proteinase, SMP or EMAP, is a homodimer aspartic protease of the peptidase A1 family. It is a non-lysosomal cathepsin, found in the membrane of gastric parietal cells, hepatic cells, proximal tubule in kidney, epithelial cells of the intestine and osteoclasts. It is also found in the endosomes of macrophages, dendritic cells, and microglia. It is involved in antigen processing on the MHC (major histocompatibility complex) class II pathway and age-related neuronal death. It has been linked to gastric and pancreatic cancers such as pancreatic ductal adenocarcinoma (PDAC), and deficiency in cathepsin E plays a role in inflammatory diseases of the skin, such as atopic dermatitis. PDAC is one of the leading causes of death in the context of cancer, mainly due to the lack of early markers of the disease. Cathepsin E is found at high levels in gastric, cervical, esophageal and lung adenocarcinomas, and others, making it an attractive biomarker and imaging peptide probe. Inhibitors that selectively act on cathepsin E and not on cathepsin D have been difficult to identify. Future studies are required to delineate the exact role of cathepsin E in cancer and to develop corresponding therapeutic approaches.

## Applications

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

**Supplied Materials**

Catalog #	Name	Amount	Storage
11070	Cathepsin E, His-Tag*	>1 µg	-80°C
	CS Substrate 1	2 x 12.5 µl	-20°C
	4x Cathepsin Buffer	2 x 2 ml	-20°C
	0.5 M DTT	2 x 200 µl	-20°C
	10 mM Pepstatin A	5 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  $\lambda_{ex}330/\lambda_{em}390$  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.
  - We recommend maintaining the diluted protein on ice during use.
  - For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](http://bpsbioscience.com).
  - We recommend using Pepstatin A 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<sub>50</sub> value shown in the validation data below.
1. Add 120 µl of **0.5 M DTT** to each 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 E**, on ice. Briefly spin the tube to recover the full content.
  4. Dilute Cathepsin E to 0.05 ng/µl with 1x Cathepsin Buffer (10 µl/well).

5. Prepare the Test Inhibitor (2.5  $\mu$ 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 25  $\mu$ 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 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  $\mu$ l of diluted Cathepsin E to all wells, except the "Negative Control" wells.
7. Add 10  $\mu$ l of 1x Cathepsin Buffer to the "Negative Control" wells.
8. Prepare the Inhibitor Control by diluting Pepstatin A (10 mM) 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  $\mu$ l/well). Using Diluent Solution prepare solutions at 1X and 10X the  $IC_{50}$  value (2.5  $\mu$ l/well).
9. Add 2.5  $\mu$ l of inhibitor solution to each well designated "Test Inhibitor".
10. Add 2.5  $\mu$ l of Diluent Solution to the "Positive Control" and "Negative Control" wells.
11. Add 2.5  $\mu$ l of diluted Pepstatin A to the "Control Inhibitor" wells.
12. Preincubate the inhibitors with the diluted Cathepsin E for 30 minutes at Room Temperature (RT) with gentle agitation.
13. Dilute 200-fold the CS Substrate 1 with 1x Cathepsin Buffer (12.5  $\mu$ l/well).
14. Initiate the reaction by adding 12.5  $\mu$ l of the diluted CS Substrate 1 to all wells. Protect your samples from direct exposure to light.
15. Incubate at RT for 30-60 minutes or perform kinetic analysis.

16. Read fluorescence intensity of the samples ( $\lambda_{\text{excitation}} = 330 \text{ nm}$ ;  $\lambda_{\text{emission}} = 390 \text{ nm}$ ) in a fluorescence microplate reader.

Component	Negative Control	Positive Control	Control Inhibitor	Test Inhibitor
1x Cathepsin Buffer	10 $\mu\text{l}$	-	-	-
Test Inhibitor	-	-	-	2.5 $\mu\text{l}$
Diluent Solution	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	-	-
Diluted Pepstatin A (1 $\mu\text{M}$ )	-	-	2.5 $\mu\text{l}$	-
Diluted Cathepsin E (0.05 ng/ $\mu\text{l}$ )	-	10 $\mu\text{l}$	10 $\mu\text{l}$	10 $\mu\text{l}$
30 minutes at Room Temperature				
Diluted CS Substrate 1 (diluted 200-fold)	12.5 $\mu\text{l}$	12.5 $\mu\text{l}$	12.5 $\mu\text{l}$	12.5 $\mu\text{l}$
<b>Total</b>	<b>25 <math>\mu\text{l}</math></b>	<b>25 <math>\mu\text{l}</math></b>	<b>25 <math>\mu\text{l}</math></b>	<b>25 <math>\mu\text{l}</math></b>

### Example Results

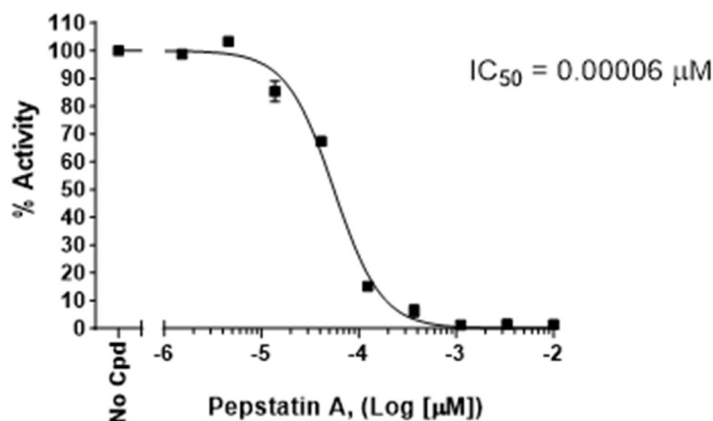


Figure 1. Inhibition of Cathepsin E activity by Pepstatin A.

Cathepsin E activity was measured in the presence of increasing concentrations of Pepstatin A. Results are expressed as percent of control (Cathepsin E 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](mailto:support@bpsbioscience.com)

### Troubleshooting Guide

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com)

### References

Pontious C., et al., 2019 *Pancreatology* 19 (7): 951-956.

**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 011524*