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 96-well format, with enough recombinant human Cathepsin E (amino acids 18-396), its substrate, and Cathepsin buffer for 96 reactions.

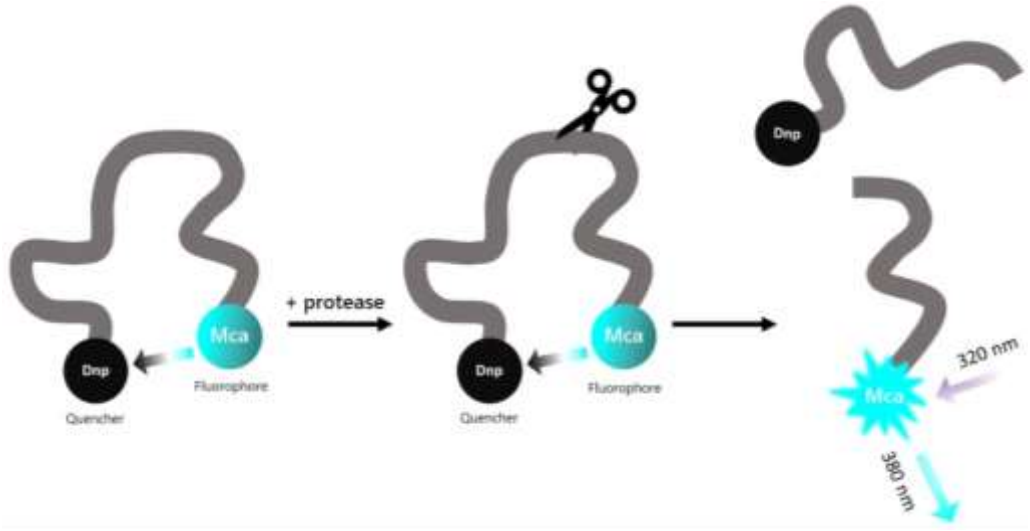

Figure 1: Illustration of the assay principle. The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent Mca from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

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 was 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.

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Supplied Materials

Catalog #	Name	Amount	Storage
11070	Cathepsin E, His-Tag*	> 1 µg	-80°C
	CS Substrate 1	12.5 µl	-80°C
	4x Cathepsin Buffer	2 ml	-20°C
	0.5 M DTT	200 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 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" 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 E**, on ice. Briefly spin the tube to recover the full content.

4. Dilute Cathepsin E to 0.05 ng/µl with 1x Assay 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 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 50 µl.

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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 µl of diluted Cathepsin E to all wells except the "Negative Control".

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

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

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

10. Dilute 200-fold the CS Substrate 1 in 1x Cathepsin Buffer.

11. Add 25 µl of the diluted CS Substrate 1 to all wells. Protect your samples from direct exposure to light.

12. Incubate at RT for 30-60 minutes or perform kinetic analysis.

13. Read fluorescence intensity of the samples (λexcitation = 330 nm; λemission = 390 nm) in a fluorescence microplate reader.

Component	**Negative control**	**Positive Control**	**Test Inhibitor**
1x Cathepsin Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted Cathepsin E (0.05 ng/µl)	-	20 µl	20 µl
Diluted CS Substrate 1 (diluted 200-fold)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

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Example Results

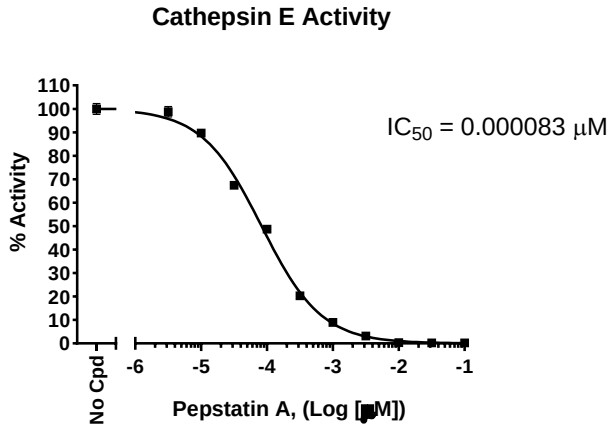

Figure 1. Inhibition of Cathepsin E activity by pepstatin A.
Cathepsin E activity was measured in the presence of increasing concentrations of pepstatin A (Santa Cruz Bio #sc-45036). The Blank value was subtracted from all other values. Results are expressed as percent of control (Cathepsin E activity 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

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

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

Related Products

Products	*Catalog #*	*Size*
Cathepsin D, His-Tag Recombinant	101391	10 µg
BACE1 Assay Kit	71656	96 reactions
BACE1, His-Tag (Human) Recombinant	71657	25 µg/100 µg

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