Description

The Cathepsin C (DPPI) Inhibitor Screening Assay Kit is a fluorogenic assay designed to measure the protease activity of human Cathepsin C, also known as DPPI (dipeptidyl peptidase I) for screening and profiling applications. This kit comes in a convenient 96-well format, with enough recombinant human Cathepsin C (amino acids 25-463, procathepsin C), Cathepsin L (amino acids 18-333) for activation, substrate and buffer for 100 reactions.

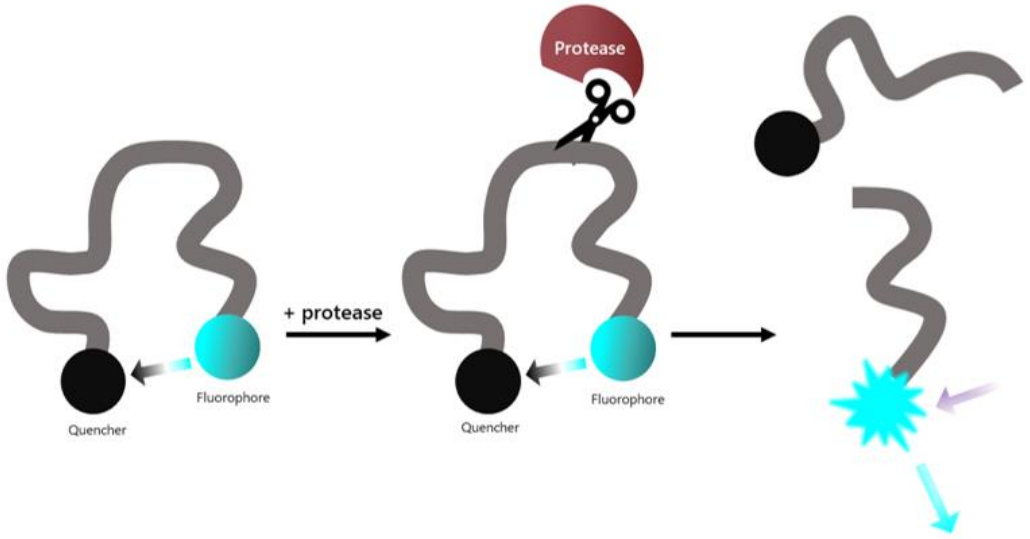

Figure 1: Illustration of the assay principle.
The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent substrate from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

Cathepsin C, also known as DPPI (dipeptidyl peptidase I), is a lysosomal aspartic protease with dipeptidylpeptidase activity over a broad range of substrates. It is mostly found in the lysosomes, where it degrades intra- and extra-cellular proteins, regulates cell death, and plays a role in inflammation. It can activate proteins such as elastase, and granzyme A and B. It is found at high levels in several types of cancer, and it has been linked to an increase in metastasis and angiogenesis. Its role in cancer has made it an attractive target in oncology, with active development of specific, membrane permeable inhibitors.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
83612-KC30	Cathepsin C/DPPI, His-Tag*	30 µg	-80°C
80005	Cathepsin L, His-tag*	10 µg	-80°C
83785-KC5	CS Substrate 3	5 µl	-80°C
78169-KC2	4x Cathepsin Buffer	2 x 2 ml	-80°C
82735-KC200	0.5 M DTT	2x 200 µl	-80°C
79685	96-well black microplate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescence plate reader capable of measurement at λex350/λem450 nm.

Storage Conditions

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.

Contraindications

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control”, 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 Brensocatib (#83748) as an internal control. If not running a dose response curve for the control inhibitor, we recommend running it at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Cathepsin C Activation

1. Thaw **4x Cathepsin Buffer** and **0.5 M DTT**.

2. Add 120 µl of **0.5 M DTT** to **4x Cathepsin Buffer**.

   Note: To prepare smaller amounts dilute 0.5 M DTT 25-fold with 4x Cathepsin Buffer to prepare a 20 mM DTT solution.

3. Prepare a 4-fold dilution of 4x Cathepsin Buffer (containing DTT) with distilled water. This makes **1x Assay Buffer**.

4. Thaw **Cathepsin C** and **Cathepsin L** on ice. Briefly spin the tubes to recover the full content.

5. Prepare a mixture containing **Cathepsin C** diluted to 0.1 mg/ml and **Cathepsin L** diluted to 0.033 mg/ml in 1x Assay Buffer (2.5 µl/well).

   Note: You will need 300 µl for a full plate or at least 2.5 µl/well.

6. Incubate the mixture at Room Temperature (RT) for 1 hour. This results in **activated Cathepsin C**.

7. Proceed immediately to next steps.

Step 2: Reaction

1. Dilute **activated Cathepsin C** 8-fold to 12.5 ng/µl with **1x Assay Buffer** (20 µl/well).

2. Add 20 µl of **diluted activated Cathepsin C** to all wells, except "Blank" wells.

3. Add 20 µl of **1x Assay Buffer** to "Blank" wells.

4. 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.

   4.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   4.2. If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay 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%.

5. Add 5 µl of **Test Inhibitor** to each well labeled as "Test Inhibitor".

6. Add 5 µl of **Diluent Solution** to the "Positive Control" and "Blank" wells.

7. Cover the plate and pre-incubate at RT for 30 minutes.

8. Dilute 500-fold the **CS Substrate 3** with **1x Assay Buffer** (25 µl/well).

9. Add 25 µl of **diluted CS substrate 3** to all wells.

10. Incubate at RT for 1 hour.

11. Read fluorescence intensity of the samples (λexcitation = 350 nm; λemission = 450 nm) in a fluorescence microplate reader.

	Blank	**Positive Control**	**Test Inhibitor**
Diluted Activated Cathepsin C (12.5 ng/µl)	-	20 µl	20 µl
1x Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
After 30 minutes of pre-incubation at RT			
Diluted CS Substrate 3	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

Example Results

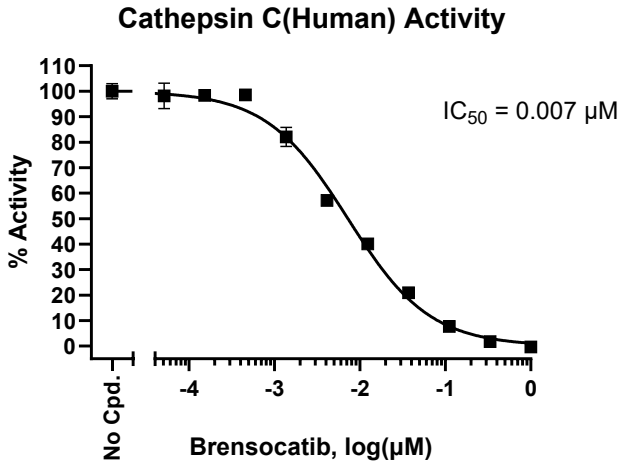

Figure 2: Inhibition of Cathepsin C by Brensocatib.
Activated Cathepsin C was incubated with increasing concentrations of Brensocatib (#83748). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

References

Zhao K., *et al.*, 2024 *Biomarker Research* 12:165.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Cathepsin E Inhibitor Screening Assay Kit	82110	96 reactions/ 384 reactions
Cathepsin D Inhibitor Screening Assay Kit	82141	96 reactions/ 384 reactions
Cathepsin L Inhibitor Screening Assay Kit	79591	96 reactions/ 384 reactions
Cathepsin B Inhibitor Screening Assay Kit	79590	96 reactions/ 384 reactions
Cathepsin S Inhibitor Screening Assay Kit	79588	96 reactions/ 384 reactions

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