**Description**

The Cathepsin D Inhibitor Screening Assay Kit is designed to measure the protease activity of Cathepsin D for screening and profiling applications. The Cathepsin D assay kit comes in a convenient 384-well format, with enough recombinant human Cathepsin D (amino acids 21-412), its substrate, and Cathepsin buffer for 384 reactions. This kit contains Pepstatin A as internal control.

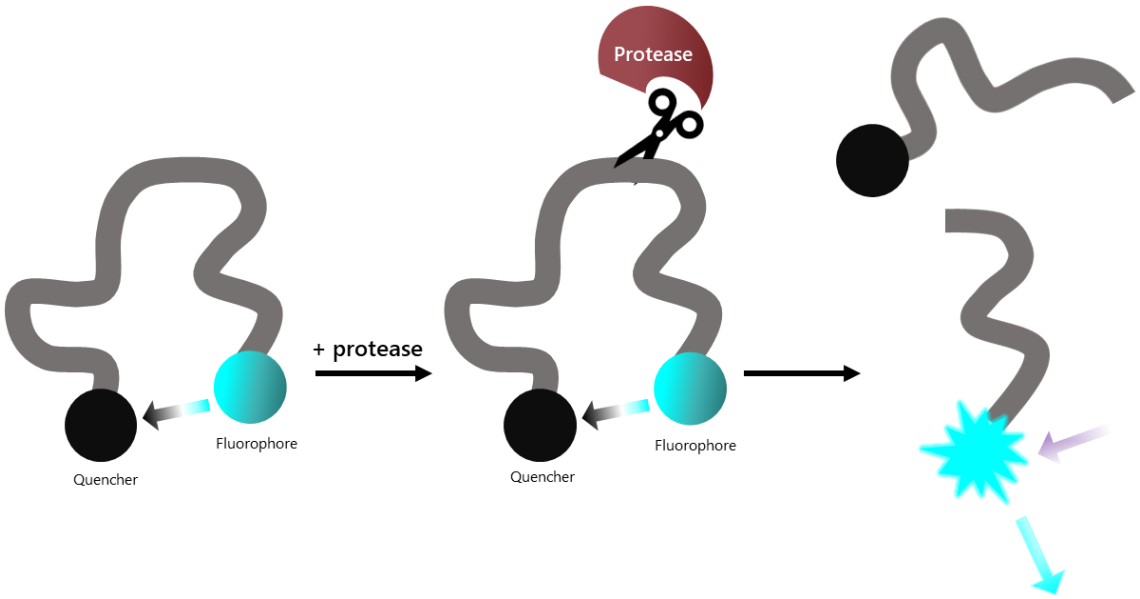


*Figure 1: Illustration of the assay principle.*
The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent substrate from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

**Background**

Cathepsin D is a lysosomal aspartyl protease, of the peptidase A1 family. It is involved in lysosomal protein degradation and activation of proteins that are synthesized as precursors, such as hormones and growth factors. Its activity impacts cell death and inflammation. Cathepsin D dysfunction has been linked to breast and gastric cancer, Alzheimer's disease (AD) and neuronal ceroid lipofuscinosis (NCL). Overexpression of this protein can lead to activation of VEGF-C (vascular endothelial growth factor -C) and VEGF-D and metastasis and angiogenesis. Cathepsin D is therefore a promising new therapeutic target. It has been shown that an antibody against Cathepsin D was able to inhibit growth of triple-negative breast cancer cells. Cathepsin D inhibition is also a promising approach in combination treatments, by maximizing the effect of other anti-cancer drugs.

**Applications**

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

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 101391 | Cathepsin D, His-Tag* | 2 µg | -80°C |
| | CS Substrate 2 | 2 x 12.5 µl | -20°C |
| | 4x Cathepsin Buffer | 2 x 2 ml | -20°C |
| | 0.5 M DTT | 2x 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 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", "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 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 $IC_{50}$ 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 D**, on ice. Briefly spin the tube to recover the full content.

4. Dilute Cathepsin D to 0.25 ng/µl with 1x Cathepsin Buffer (10 µl/well).

5. Prepare the Test Inhibitor (2.5 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 25 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 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 10 µl of diluted Cathepsin D to all wells except the "Negative Control".

7. Add 10 µl 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 µl/well). Using Diluent Solution prepare solutions at 1X and 10 X the $IC_{50}$ value (2.5 µl/well).

9. Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor".

10. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

11. Add 2.5 µl of diluted Pepstatin A to the "Control Inhibitor" wells.

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

13. Dilute 200-fold the CS Substrate 2 with 1x Cathepsin Buffer.

14. Add 12.5 µl of the diluted CS Substrate 2 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 (λexcitation = 330 nm; λemission = 390 nm) in a fluorescence microplate reader.

| Component | Negative Control | Positive Control | Control Inhibitor | Test Inhibitor |
|---|---|---|---|---|
| 1x Cathepsin Buffer | 10 µl | - | - | - |
| Test Inhibitor | - | - | - | 2.5 µl |
| Diluent Solution | 2.5 µl | 2.5 µl | - | - |
| Diluted Pepstatin A | - | - | 2.5 µl | - |
| Diluted Cathepsin D (0.25 ng/µl) | - | 10 µl | 10 µl | 10 µl |
| 30 minutes at Room Temperature | | | | |
| Diluted CS Substrate 2 (diluted 200-fold) | 12.5 µl | 12.5 µl | 12.5 µl | 12.5 µl |
| **Total** | **25 µl** | **25 µl** | **25 µl** | **25 µl** |

**Example Results**

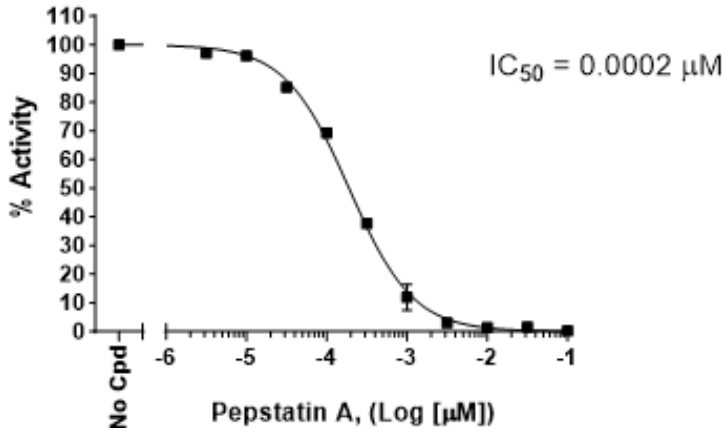


*Figure 1. Inhibition of Cathepsin D activity by Pepstatin A.*
Cathepsin D activity was measured in the presence of increasing concentrations of Pepstatin A. The Blank value was subtracted from all other values. Results are expressed as percent of control (Cathepsin D activity in the absence of inhibitor, 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**
Seo S., *et al.*, 2022 *Cell Death and Disease* 13: 115.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| Cathepsin E, His-Tag Recombinant | 11070 | 10 µg |
| Cathepsin B, His-tag Recombinant | 80001 | 10 µg |
| Cathepsin E Inhibitor Screening Assay Kit | 82110 | 96 reactions/384 reactions |
| Cathepsin B Inhibitor Screening Assay Kit | 79590 | 96 reactions/384 reactions |

*Version 120723*