Description

The Cathepsin S Inhibitor Screening Assay Kit is a homogeneous fluorogenic assay designed to measure the protease activity of Cathepsin S for screening and profiling applications. The Cathepsin S assay kit comes in a convenient 96-well format, with enough purified Cathepsin S (amino acids 17-331), its substrate, and Cathepsin Buffer for 100 reactions. This kit includes the inhibitor E-64 as control.

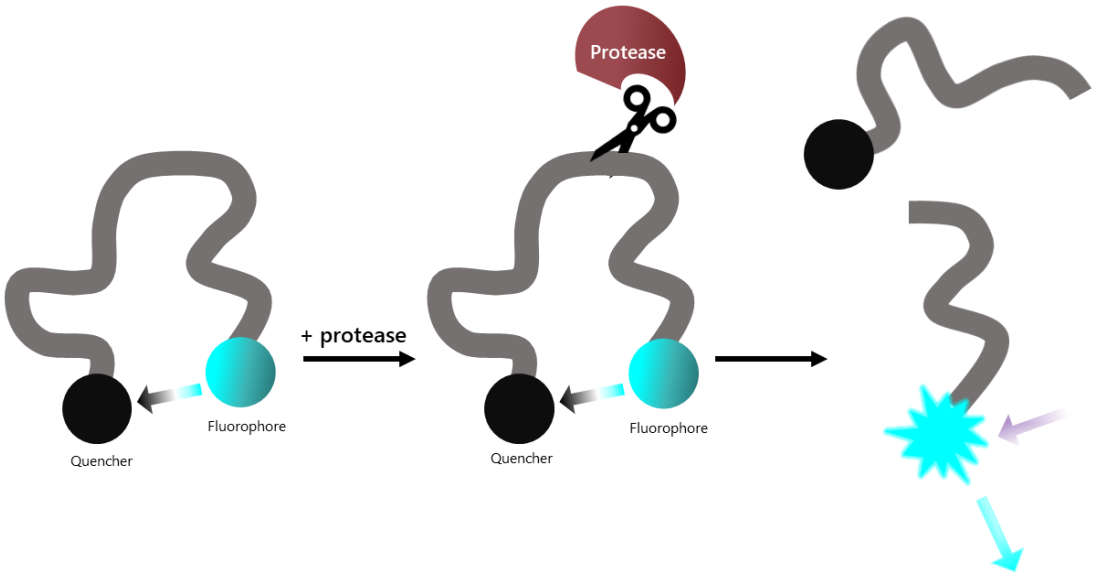

Figure 1: Illustration of the assay principle.
The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent substrate from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

Cathepsin S is a cysteine protease of the C1 papain family, found inside the lysosomal/endosomal compartments of antigen-presenting cells such as B cells, macrophages, and dendritic cells. Cathepsin S participates in the degradation of antigenic proteins to peptides for presentation. Contrary to other cathepsins, Cathepsin S functions at neutral pH and it is secreted in response to inflammatory triggers. Its activity is regulated by cystacin C, its endogenous inhibitor. Cathepsin S has additional biological effects, acting as one of the most potent elastases, cleaving a number of extracellular matrix (ECM) proteins. Its role as elastase contributes to blood vessel permeability and angiogenesis. High levels of this protein can be found in psoriatic keratinocytes and pulmonary fibrosis, and it has also been linked to nociception. The development of inhibitors targeting Cathepsin S can prove beneficial in the treatment of pulmonary fibrosis and other Cathepsin S-linked diseases.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80008-KC4	Cathepsin S, His-Tag*	4 µg	-80°C
80349	5 mM Fluorogenic Cathepsin Substrate 1	10 µl	-20°C
78169-KC2	4x Cathepsin Buffer	2 ml	-20°C
82725-KC200	0.5 M DTT	200 µl	-20°C
82816-KC10	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.*

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.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

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 S**, on ice. Briefly spin the tube to recover the full content.

4. Dilute **Cathepsin S** to 1 ng/µl in 1x Cathepsin Buffer (20 µl/well).

   Note: Intermediate dilution of Cathepsin S might be required. For example, dilute first Cathepsin S to 10 ng/µl with 1x Cathepsin Buffer and then dilute further to 1 ng/µl using 1x Cathepsin Buffer.

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.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions of the inhibitor 10-fold more concentrated 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 S** 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** following the guidelines provided for the Test inhibitor (see above).

   Note: E-64 is soluble in DMSO.

9. Add 5 µl of **Test 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 **Inhibitor Control** solution to the "Control Inhibitor" wells.

12. Preincubate the plate for 30 minutes at Room Temperature (RT) with gentle agitation.

13. Dilute 500-fold the **5 mM 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 the 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 S (1 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

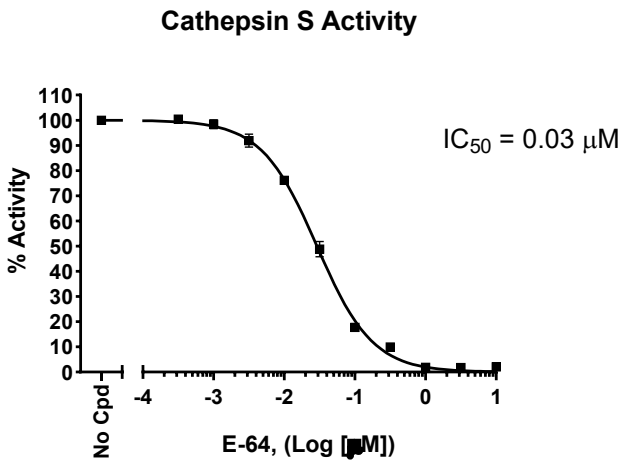

Figure 2: Inhibition of Cathepsin S activity by E-64.
Cathepsin S activity was measured in the presence of increasing concentrations of E-64. Results are expressed as percent of control (Cathepsin S activity in the absence of inhibitor, set at 100%).

Data shown is representative.

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.

References
Yoo Y., *et al.*, 2022 *Biomedicine & Pharmacotherapy* 145: 112245.

Related Products

Products	*Catalog #*	*Size*
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

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