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**Data sheet**  
***Cathepsin F Inhibitor Screening Assay Kit***  
Catalog #79971  
Size: 96 reactions

**BACKGROUND:** Cathepsin F is a lysosomal cysteine protease that belongs to the papain-like superfamily. It is secreted by macrophages and is thought to play a role in processing and loading peptides into MHC complexes. Cathepsin F is also expressed in atherosclerotic lesions, where it degrades ApoB-100, triggering LDL aggregation. It has been implicated in tumor invasion and metastasis, inducing apoptosis of gastric cancer cells, and mutations in cathepsin F have been linked to Alzheimer's disease and Type B Kuf's disease.

**DESCRIPTION:** The *Cathepsin F Inhibitor Screening Assay Kit* is designed to measure the protease activity of Cathepsin F for screening and profiling applications. The Cathepsin F assay kit comes in a convenient 96-well format, with purified Cathepsin F, its fluorogenic substrate, and Cathepsin buffer for 100 enzyme reactions.

**COMPONENTS:**

| Catalog # | Component                                  | Amount | Storage   |                                    |
|-----------|--|--------|-----------|------------------------------------|
| 80003     | Cathepsin F                                | >1 µg  | -80°C     | Avoid multiple freeze/thaw cycles! |
| 80349     | Fluorogenic Cathepsin F Substrate (0.5 mM) | 100 µl | -20°C     |                                    |
|           | *4X Cathepsin buffer                       | 2 ml   | -20°C     |                                    |
| 79685     | 96-well black microplate                   | 1      | Room Temp |                                    |

\*Add 120 µl of 0.5 M DTT before use

**APPLICATIONS:** Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

**STABILITY:** Up to 6 months from date of receipt, when stored as recommended

**REFERENCE:**

Smith, Katherine R., *et al.* 2013. "Cathepsin F mutations cause Type B Kufs disease, an adult-onset neuronal ceroid lipofuscinosis." *Human Molec. Genetics* **22(7)**: 1417-1423.

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

0.5 M DTT in aqueous solution  
Adjustable micropipettor and sterile tips  
Fluorescent microplate reader

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#### ASSAY PROTOCOL:

*All samples and controls should be tested in duplicate.*

- 1) Add 120  $\mu$ l of 0.5 M DTT to **4x Cathepsin buffer**. Prepare **1x Cathepsin buffer** by diluting **4x Cathepsin buffer** 4-fold into water. Prepare only the amount required for the assay; store remaining 4x cathepsin buffer as directed.
- 2) Add 20  $\mu$ l **1X Cathepsin buffer** to each well designated "Blank."
- 3) Thaw **Cathepsin F** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Prepare an intermediate Cathepsin F solution by diluting the enzyme to 10 ng/ $\mu$ l in **1X Cathepsin buffer**. Aliquot remaining **Cathepsin F** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at  $-80^{\circ}\text{C}$ . Note: **Cathepsin F** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
- 4) Prepare **Cathepsin F** (0.5 ng/ $\mu$ l) by diluting in **1X Cathepsin buffer**.
- 5) Add 20  $\mu$ l **Cathepsin F** (0.5 ng/ $\mu$ l) to each well designated "Positive Control" and "Test Inhibitor."
- 6) Add 5  $\mu$ l **Inhibitor solution** to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5  $\mu$ l of the same solution without inhibitor ("Inhibitor buffer", usually 10% DMSO in water).

*Note: Final DMSO concentration must be  $\leq 1\%$ . Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10  $\mu\text{M}$ , dilute 1 mM inhibitor with water to make a 100  $\mu\text{M}$  inhibitor in 10% DMSO(aq). Then, add 5  $\mu$ l of the 100  $\mu\text{M}$  solution into the 50  $\mu$ l assay to make a 1% DMSO concentration in the final reaction mixture.*

- 7) Prepare **Substrate solution** (20  $\mu\text{M}$ ) by diluting **Fluorogenic Cathepsin substrate 1** (0.5 mM) in **1X Cathepsin buffer**. Store remaining undiluted substrate in aliquots at  $-20^{\circ}\text{C}$ . Do not re-use diluted substrate.

|  | Positive Control | Test Inhibitor | Blank      |
|--|------------------|----------------|------------|
| Cathepsin F (0.5 ng/ $\mu$ l)          | 20 $\mu$ l       | 20 $\mu$ l     | -          |
| 1X Cathepsin buffer                    | -                | -              | 20 $\mu$ l |
| Inhibitor (in Cathepsin buffer)        | -                | 5 $\mu$ l      | -          |
| 10% DMSO in water (Inhibitor buffer)   | 5 $\mu$ l        | -              | 5 $\mu$ l  |
| Substrate solution (20 $\mu\text{M}$ ) | 25 $\mu$ l       | 25 $\mu$ l     | 25 $\mu$ l |
| Total                                  | 50 $\mu$ l       | 50 $\mu$ l     | 50 $\mu$ l |

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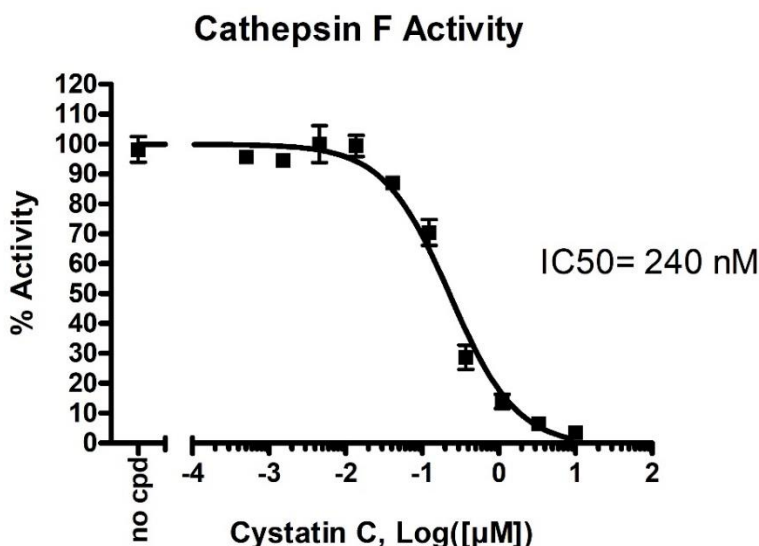
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- 8) Add 25  $\mu$ l **Substrate solution** (20  $\mu$ M) to all wells. Incubate reaction at room temperature for 60 minutes.
- 9) Read fluorescence intensity of the samples ( $\lambda_{excitation}$  = 360 nm;  $\lambda_{emission}$  = 460 nm) in an appropriate microplate reader. "Blank" value is subtracted from all readings.

**Example of assay results:**



Cathepsin F inhibition by Cystatin C, measured using the *Cathepsin F Inhibitor Screening Assay Kit*, BPS Bioscience, #79971. Fluorescence was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

**RELATED PRODUCTS:**

| <u>Product</u>                            | <u>Catalog#</u> | <u>Size</u> |
|---|-----------------|-------------|
| Cathepsin B                               | 80001           | 10 $\mu$ g  |
| Cathepsin F                               | 80003           | 10 $\mu$ g  |
| Cathepsin L                               | 80005           | 10 $\mu$ g  |
| Cathepsin S                               | 80008           | 10 $\mu$ g  |
| Cathepsin V                               | 80009           | 10 $\mu$ g  |
| Fluorogenic Cathepsin Substrate 1         | 80349           | 100 $\mu$ l |
| Fluorogenic Cathepsin F Substrate         | 80350           | 100 $\mu$ l |
| Cathepsin B Inhibitor Screening Assay Kit | 79590           | 96 rxns.    |
| Cathepsin L Inhibitor Screening Assay Kit | 79591           | 96 rxns.    |
| Cathepsin S Inhibitor Screening Assay Kit | 79588           | 96 rxns.    |
| Cathepsin V Inhibitor Screening Assay Kit | 79589           | 96 rxns.    |

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