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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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 µl 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 µl **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/µl in **1X Cathepsin buffer**. Aliquot remaining **Cathepsin F** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°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/µl) by diluting in **1X Cathepsin buffer**.
5) Add 20 µl **Cathepsin F** (0.5 ng/µl) to each well designated "Positive Control" and "Test Inhibitor."
6) Add 5 µl **Inhibitor solution** to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5 µl of the same solution without inhibitor ("Inhibitor buffer", usually 10% DMSO in water).

   Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.
7) Prepare **Substrate solution** (20 µM) by diluting **Fluorogenic Cathepsin substrate 1** (0.5 mM) in **1X Cathepsin buffer**. Store remaining undiluted substrate in aliquots at -20°C. Do not re-use diluted substrate.

	Positive Control	**Test Inhibitor**	**Blank**
Cathepsin F (0.5 ng/µl)	20 µl	20 µl	-
1X Cathepsin buffer	-	-	20 µl
Inhibitor (in Cathepsin buffer)	-	5 µl	-
10% DMSO in water (Inhibitor buffer)	5 µl	-	5 µl
Substrate solution (20 µM)	25 µl	25 µl	25 µl
Total	50 µl	50 µl	50 µl

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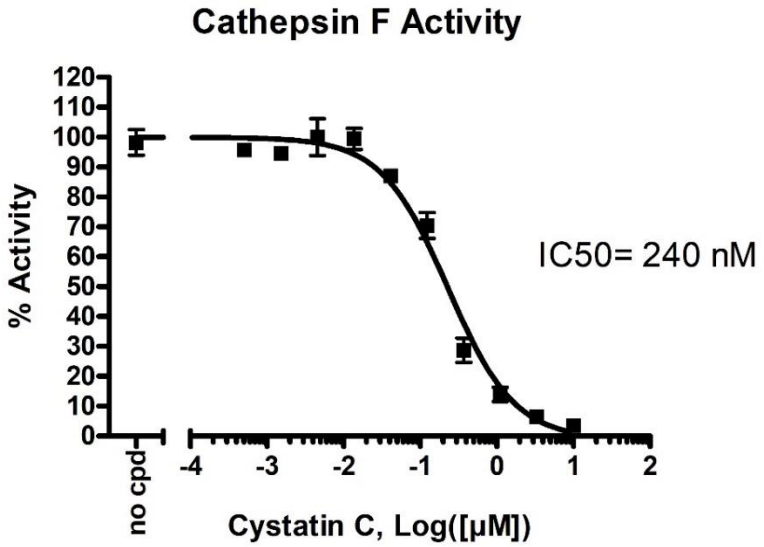
8) Add 25 µl **Substrate solution** (20 µM) to all wells. Incubate reaction at room temperature for 60 minutes.

9) Read fluorescence intensity of the samples (λexcitation = 360 nm; λemission = 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

RELATED PRODUCTS:

Product	Catalog#	Size
Cathepsin B	80001	10 µg
Cathepsin F	80003	10 µg
Cathepsin L	80005	10 µg
Cathepsin S	80008	10 µg
Cathepsin V	80009	10 µg
Fluorogenic Cathepsin Substrate 1	80349	100 µl
Fluorogenic Cathepsin F Substrate	80350	100 µl
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.