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Data sheet

Cathepsin L Inhibitor Screening Assay Kit

Catalog #79591
Size: 96 reactions

BACKGROUND: Cathepsin L is a lysosomal cysteine protease that belongs to the papain-like superfamily. It is involved in many essential physiological processes, including degradation and renewal of intracellular proteins, activation of prohormones, and presentation of antigens, as well as organ development. Cathepsin L is known to play important roles in tumor metastasis and chemotherapy resistance, and upregulation of this enzyme has been reported in a wide range of human cancers including ovarian, renal, and breast carcinoma.

DESCRIPTION: The *Cathepsin L Inhibitor Screening Assay Kit* is designed to measure the protease activity of Cathepsin L for screening and profiling applications. The Cathepsin L assay kit comes in a convenient 96-well format, with purified Cathepsin L, its fluorogenic substrate, and Cathepsin buffer for 100 enzyme reactions. In addition, the kit includes the cathepsin inhibitor E-64 for use as a control inhibitor.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80005	Cathepsin L	10 µg	-80 °C	Avoid multiple freeze/thaw cycles!
80349	Fluorogenic Cathepsin Substrate 1 (5 mM)	10 µl	-20 °C	
	*4X Cathepsin buffer	2 ml	-20 °C	
	E-64 (1 mM)	10 µl	-20 °C	
79685	96-well black microplate			

*Add 120 µl of 0.5 M DTT before use

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
0.5 M DTT in aqueous solution
Adjustable micropipettor and sterile tips
Fluorescent microplate reader

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.

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REFERENCES:

Han, M. L., *et al.* (2016). Cathepsin L upregulation-induced EMT phenotype is associated with the acquisition of cisplatin or paclitaxel resistance in A549 cells. *Acta Pharmacologica Sinica,* **37(12),** 1606.

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.
2) Add 20 µl **1X Cathepsin buffer** to each well designated "Blank".
3) Thaw **Cathepsin L** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Prepare an intermediate Cathepsin L solution by diluting the enzyme to 10 ng/µl in **1X Cathepsin buffer**. Aliquot remaining **Cathepsin L** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **Cathepsin L** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
4) Prepare **Enzyme solution** (0.02 ng/µl Cathepsin L) by diluting **10 ng/µl Cathepsin L** 500-fold in **1X Cathepsin buffer**. For example, to prepare 1000 µl, add 2 µl **10 ng/µl Cathepsin L** to 998 µl **1X Cathepsin buffer**.
5) Add 20 µl **Enzyme solution** (0.02 ng/µl Cathepsin L) to each well designated "Positive Control", "Negative 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 **Inhibitor buffer** (same solution without inhibitor). Optional: for each well designated "Negative Control", add 5 µl **E-64** diluted 0.1 – 0.0001 µM in **1X Cathepsin buffer**. Incubate at room temperature for 10 minutes.
7) Prepare **Substrate solution** (10 µM) by diluting **Fluorogenic Cathepsin substrate 1** (5 mM) 500-fold in **1X Cathepsin buffer**. For example, to prepare 1000 µl, add 2 µl **Fluorogenic Cathepsin Substrate 1** (5 mM) to 998 µl **1X Cathepsin buffer**. Store remaining undiluted substrate in aliquots at -20°C. Do not re-use diluted substrate.
8) Add 25 µl **Substrate solution** (10 µM) to all wells. Incubate reaction at room temperature for 60 minutes.

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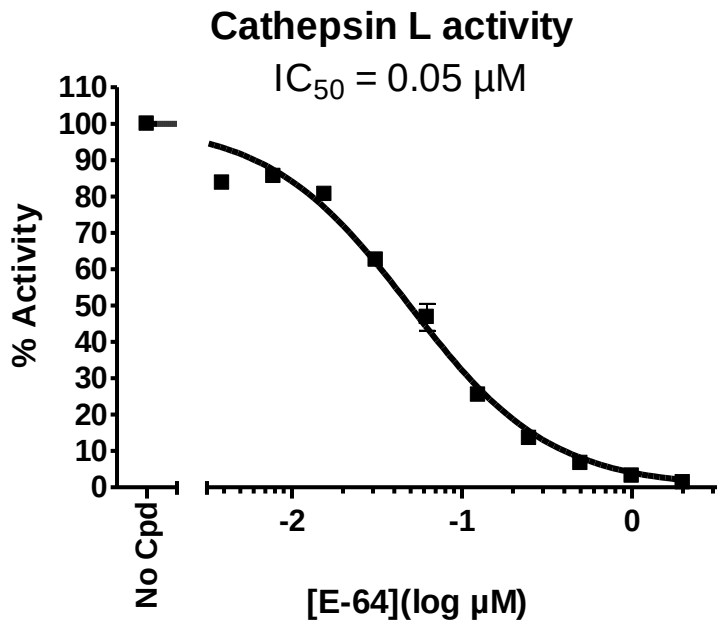
	Positive Control	Negative Control	Test Inhibitor	Blank
Enzyme solution (0.02 ng/µl Cathepsin L)	20 µl	20 µl	20 µl	-
1X Cathepsin buffer	-	-	-	20 µl
Inhibitor (in Cathepsin buffer)	-	-	5 µl	-
Inhibitor buffer (no inhibitor)	5 µl	-	-	5 µl
E-64	-	5 µl	-	
Substrate solution (10 µM)	25 µl	25 µl	25 µl	25 µl
Total	50 µl	50 µl	50 µl	50 µl

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 L inhibition by E-64, measured using the *Cathepsin L Inhibitor Screening Assay Kit*, BPS Bioscience, #79591. 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

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RELATED PRODUCTS:

Product	Catalog#	Size
Cathepsin B	80001	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

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