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Data sheet

Cathepsin B Inhibitor Screening Assay Kit

Catalog #79590
Size: 96 reactions

BACKGRUND: Cathepsin B is a cysteine protease that primarily functions as an endopeptidase within endolysosomal compartments in normal cells. High levels of cathepsin B are found in a wide variety of human cancers and in experimental models, such as transgenic models of murine pancreatic and mammary carcinomas, causal roles for this protein have been demonstrated in initiation, growth/tumor cell proliferation, angiogenesis, invasion, and metastasis.

DESCRIPTION: The *Cathepsin B Inhibitor Screening Assay Kit* is designed to measure the protease activity of Cathepsin B for screening and profiling applications. The Cathepsin B assay kit comes in a convenient 96-well format, with purified Cathepsin B, 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	
80001	Cathepsin B	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	
	96-well black microplate			

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.

REFERENCES:
1. Aggarwal, N., & Sloane, B. F. (2014). Cathepsin B: multiple roles in cancer. *PROTEOMICS–Clinical Applications* ***8*****(5-6),** 427-437.
2. Siklos, M., BenAissa, M., & Thatcher, G. R. (2015). Cysteine proteases as therapeutic targets: does selectivity matter? A systematic review of calpain and cathepsin inhibitors. *Acta Pharmaceutica Sinica B,* ***5*****(6),** 506-519.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Prepare **1X Cathepsin buffer** by diluting **4X Cathepsin buffer** 4-fold and 0.5 M DTT 100-fold (5 mM final assay concentration) into water. For example, to prepare 10 ml, add 2.5 ml of **4X Cathepsin buffer** and 0.1 ml of 0.5 M DTT to 7.4 ml of water.
2) Add 20 µl **1X Cathepsin buffer** to each well designated "Blank".
3) Thaw **Cathepsin B** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Activate **Cathepsin B** by diluting the enzyme to 10 ng/µl in **1X Cathepsin buffer** for 15 minutes at room temperature. Aliquot remaining **Cathepsin B** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **Cathepsin B** 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 B) by diluting **10 ng/µl diluted, activated Cathepsin B** 500-fold in **1X Cathepsin buffer**. For example, to prepare 1000 µl, add 2 µl **10 ng/µl activated Cathepsin B** to 998 µl **1X Cathepsin buffer**.
5) Add 20 µl **Enzyme solution** (0.02 ng/µl Cathepsin B) 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.

	Positive Control	Negative Control	Test Inhibitor	Blank
Enzyme solution (0.02 ng/µl Cathepsin B)	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

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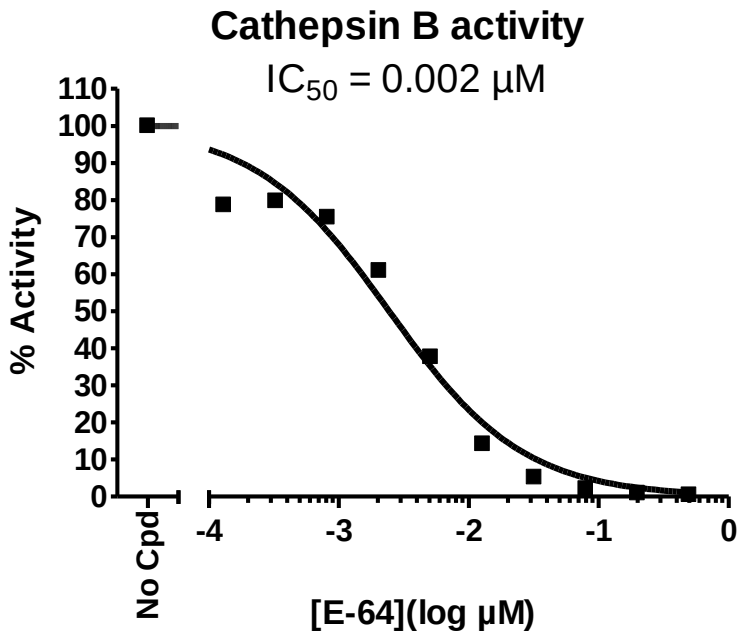
9) Read fluorescence intensity of the samples (λexcitation = 360 nm; λemission = 460 nm) in an appropriate microplate reader. "Blank" value is subtracted from all readings.

Example of assay results:



Cathepsin B inhibition by E-64, measured using the *Cathepsin B Inhibitor Screening Assay Kit*, BPS Bioscience, #79590. 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 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