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Cathepsin B Inhibitor Screening Kit (Fluorometric)

rev. 8 /14

(Catalog # K147-100; 100 assays, Store kit at -20°C)

I. Introduction:

Cathepsin B (**CTSB,** EC **3.4.22.1**) is a lysosomal cysteine proteinase that is suggested to participate in intracellular degradation and turnover of proteins. It has also been implicated in tumor invasion and metastasis. Biovision's Cathepsin B Inhibitor Screening Kit utilizes the ability of Cathepsin B to cleave the synthetic AFC based peptide substrate to release AFC, which can be easily quantified using a fluorometer or fluorescence microplate reader. In the presence of a Cathepsin B-specific inhibitor, the cleavage of the substrate is reduced/abolished resulting in decrease or total loss of the AFC fluorescence. This simple and high-throughput adaptable assay kit can be used to screen/study/characterize the potential inhibitors of Cathepsin B.

CTSB Substrate-AFC $\xrightarrow{\text{Cathepsin B}}$ Cleaved substrate + AFC (Fluorescence)

CTSB Substrate-AFC $\xrightarrow{\text{Cathepsin B + CTSB inhibitor}}$ Decrease in fluorescence/No fluorescence

II. Applications:

- Screen/study/characterize potential inhibitors of Cathepsin B.

III. Kit Contents:

Components	K147-100	Cap Code	Part Number
CTSB Reaction Buffer	15 ml	WM	K147-100-1
CTSB Reagent	100 µl	Blue	K147-100-2
Cathepsin B (human)	5 µl	Green	K147-100-3
CTSB Substrate, Ac-RR-AFC (10 mM)	0.2 ml	Brown	K147-100-4
CTSB Inhibitor (F-F-FMK, 1 mM)	20 µl	Red	K147-100-5

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer.

V. Storage & Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

VI. Reagent Preparation and Storage Conditions:

- **CTSB Reaction Buffer :** Warm CTSB Reaction Buffer to room temperature before use.
- **CTSB Reagent:** Aliquot & store at -20°C. Avoid repeated freeze/thaw.
- **Cathepsin B (human):** Add 105 µl of CTSB Reaction Buffer to the vial. Gently pipette up & down to dissolve completely. Aliquot & store at -80°C. Avoid repeated freeze/thaw.

VII. Cathepsin B Inhibitor Screening Protocol:

1. **Cathepsin B Enzyme Solution Preparation**: For each well, prepare 50 µl of Cathepsin B enzyme solution.

   48 µl CTSB Reaction Buffer
   1 µl CTSB Reagent
   1 µl Cathepsin B enzyme solution

   Mix well and add 50 µl/well into a 96-well microtiter plate.

2. **Screening Compounds, Inhibitor Control & Blank Control Preparations:** Dissolve test inhibitors into proper solvent. Dilute to 10X the desired test concentration with CTSB Reaction Buffer. Add 10 µl diluted test inhibitors (**Sample**, **S**) or CTSB Reaction Buffer into Cathepsin B enzyme containing wells (**Enzyme Control**, **EC**). For Inhibitor Control (**IC**), add 1 µl CTSB Inhibitor & 9 µl CTSB Reaction Buffer into Cathepsin B enzyme well(s). Incubate at room temperature for 10-15 min.

3. **Cathepsin B Substrate Preparation:** For each well, prepare 40 µl of the substrate solution.

   38 µl CTSB Reaction Buffer
   2 µl CTSB Substrate

   Mix & add 40 µl of Cathepsin B Substrate solution into each well. Mix well.

4. **Measurement:** Measure the fluorescence in a kinetic mode for 30-60 min. at 37°C (Ex/Em = 400/505 nm). Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

5. **Calculations**: Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2-RFU_1) values with the time ΔT (T_2-T_1).

$$\% \textbf{ Relative Inhibition} = \frac{\textbf{Slope of EC} - \textbf{Slope of S}}{\textbf{Slope of EC}} \times \textbf{100}$$

Note: Irreversible inhibitors that inhibit the Cathepsin B activity completely at the tested concentration will have ΔRFU = 0 and thus the % Relative Inhibition will be 100%.

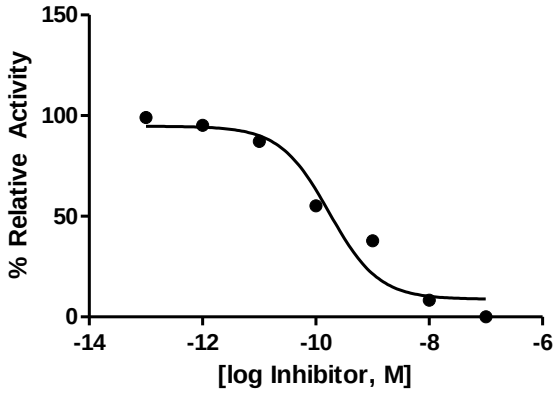

Figure: Inhibition of Cathepsin B activity by CTSB Inhibitor. Assay was performed following kit protocol.

VIII. RELATED PRODUCTS:

Cathepsin B (1021)
Cathepsin B Antibody (3190)
Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin D Antibody (3191R)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)
Cathepsin F Blocking Peptide (3371BP)
Cathepsin G Antibody (3370)
Cathepsin G Substrate (2206)
Cathepsin G Activity Fluorometric Assay Kit (K146)
Cathepsin H Activity Fluorometric Assay Kit (K145)
Procathepsin K, human recombinant (1026)
Procathepsin K, rat recombinant (1029)
Cathepsin L Activity Fluorometric Assay Kit (K142)
Cathepsin S Antibody (3366, 3366R)
Human CellExp™ Cathepsin S, human recombinant (7277)
Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin D (1022)
Cathepsin S Inhibitor Screening Kit (K149)
Cathepsin D Blocking Peptide (3191RBP)
Cathepsin F Antibody (3371)
Cathepsin G Activity Assay Kit, Fluorometric (K146)
Cathepsin G Inhibitor (1982)
Cathepsin G, human neutrophil (4713)
Cathepsin H (1023)
Cathepsin K Activity Fluorometric Assay Kit (K141)
Procathepsin K, mouse recombinant (1027)
Cathepsin K Activity Fluorometric Assay Kit (K141)
Cathepsin S Activity Fluorometric Assay Kit (K144)
Cathepsin S Blocking Peptide (3366R)

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