Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Cathepsin H Inhibitor Screening Kit (Fluorometric)

(Catalog # K164-100; 100 assays, Store kit at -20°C)

I. Introduction:

Cathepsin H (CTSH, EC 3.4.22.16), a lysosomal cysteine protease, is important in the overall degradation of lysosomal proteins. It is composed of a dimer of disulfide-linked heavy and light chains, both produced from a single protein precursor. The encoded protein, which belongs to the peptidase C1 protein family, can act both as an aminopeptidase and as an endopeptidase. Increased expression of this gene has been correlated with malignant progression of prostate tumors. BioVision's Cathepsin H Inhibitor Screening Kit utilizes the ability of an active Cathepsin H to cleave a synthetic AFC-based peptide substrate to release free AFC, which can be easily quantified using a fluorometer or fluorescence microplate reader. In the presence of a Cathepsin H-specific inhibitor, the cleavage of this 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 potential inhibitors of Cathepsin H.

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

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

II. Applications:

- Screen/study/characterize potential inhibitors of Cathepsin H

III. Kit Contents:

Components	K164-100	Cap Code	Part Number
CTSH Assay Buffer	25 ml	WM	K164-100-1
CTSH Reagent	0.1 ml	Blue	K164-100-2
Human Cathepsin H	1 vial	Green	K164-100-3
CTSH Substrate	0.2 ml	Amber	K164-100-4
CTSH Inhibitor (1 mM)	20 µl	Red	K164-100-5

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

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.

- **CTSH Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **CTSH Reagent:** Aliquot & store at -20°C. Avoid repeated freeze/thaw.
- **Human Cathepsin H:** Reconstitute in 110 µl of CTSH Assay Buffer. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

VI. Cathepsin H Inhibitor Screening Protocol:

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

   48 µl CTSH Assay Buffer
   1 µl CTSH Reagent
   1 µl Reconstituted Cathepsin H enzyme solution

   Mix well and add 50 µl/well into desired wells in 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 CTSH Assay Buffer. Add 10 µl diluted test inhibitors (Sample, S) or CTSH Assay Buffer (Enzyme Control, EC) into Cathepsin H enzyme containing wells. For Inhibitor Control (IC), add 1 µl CTSH Inhibitor and 9 µl CTSH Assay Buffer into Cathepsin H enzyme well(s). Incubate at room temperature for 15 min.

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well with the same final concentration of the solvent as in the inhibitor sample as solvent control.

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

   39 µl CTSH Assay Buffer
   1 µl CTSH Substrate

   Mix & add 40 µl of Cathepsin H Substrate solution into Enzyme Control, Inhibitor Control & sample wells. Mix well.

4. **Measurement:** Measure fluorescence (Ex/Em = 400/505 nm) in a kinetic mode for 1-2 hr at 37°C. Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

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 H activity completely at the tested concentration will have ΔRFU = 0 and thus the % Relative Inhibition will be 100%.

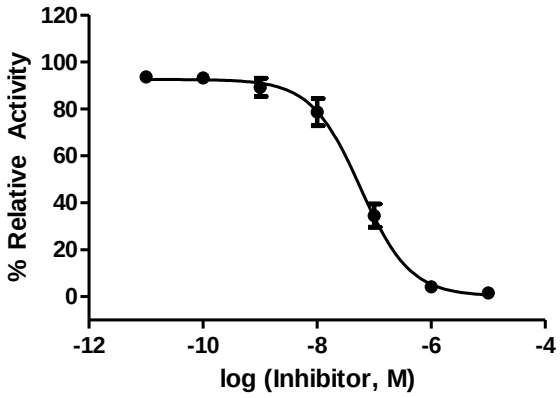

Figure: Inhibition of Cathepsin H activity by CTSH Inhibitor. Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Cathepsin L Activity Fluorometric Assay Kit (K142)
Cathepsin L (Cleaved) Antibody (3741)
Cathepsin L Blocking Peptide (3192BP)
Cathepsin B (1021)
Cathepsin B Antibody (3190)
Cathepsin D (1022)
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 K Blocking Peptide (3588BP, 3368BP)
Cathepsin S Activity Fluorometric Assay Kit (K144)
Cathepsin S Antibody (3366, 3366R)
Cathepsin L Antibody (3192)
Cathepsin L (Cleaved) Blocking Peptide (3741BP)
Cathepsin L, human recombinant (1135)
Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin B Inhibitor Screening Kit (K147)
Cathepsin D Activity Fluorometric Assay Kit (K143)
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 Antibody (3588, 3368)
Human CellExp™ Cathepsin S, human recombinant (7277)
Cathepsin S Inhibitor Screening Kit (K149)
Cathepsin S Blocking Peptide (3366R)

FOR RESEARCH USE ONLY! Not to be used on humans.