



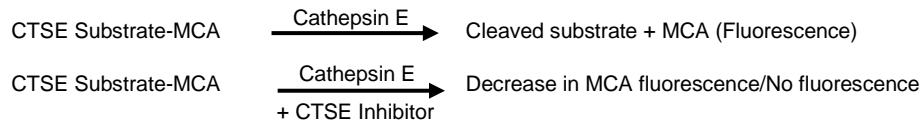
Cathepsin E Inhibitor Screening Kit (Fluorometric)

5/15

(Catalog # K166-100; 100 assays, Store kit at -20°C)

I. Introduction:

Cathepsin E (CTSE, EC: 3.4.23.34) is a gastric aspartyl protease that functions as a disulfide-linked homodimer. This protease has a specificity similar to that of pepsin A and cathepsin D. It is an intracellular proteinase that is found in highest concentration on the surface of epithelial mucus-producing cells of the stomach. It is the first aspartic proteinase expressed in the fetal stomach and is found in more than half of the gastric cancers. BioVision's Cathepsin E Inhibitor Screening Kit utilizes the ability of Cathepsin E to cleave a synthetic MCA-based peptide substrate to release free MCA, which can be easily quantified using a fluorometer or fluorescence microplate reader. In the presence of a Cathepsin E-specific inhibitor, the cleavage of the substrate is reduced/abolished resulting in decrease or total loss of the MCA fluorescence. This simple and high-throughput adaptable assay kit can be used to screen/study/characterize the potential inhibitors of Cathepsin E.



II. Applications:

- Screening/studying/characterizing inhibitors of Cathepsin E.

III. Kit Contents:

Components	K166-100	Cap Code	Part Number
CTSE Assay Buffer	25 ml	NM	K166-100-1
Human Cathepsin E	1 vial	Green	K166-100-2
CTSE Substrate	0.2 ml	Brown	K166-100-3
CTSE Inhibitor (1 mM Pepstatin A in DMSO)	20 µl	Blue	K166-100-4

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.

- **CTSE Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **Human Cathepsin E:** Reconstitute in 220 µl of de-ionized water. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months.

VI. Cathepsin E Inhibitor Screening Protocol:

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

48 µl CTSE Assay Buffer
2 µl Reconstituted Cathepsin E 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 CTSE Assay Buffer. Add 10 µl diluted test inhibitors (Sample, S) or CTSE Assay Buffer (Enzyme Control, EC) into Cathepsin E enzyme containing wells. For Inhibitor Control (IC), add 1 µl CTSE Inhibitor and 9 µl CTSE Assay Buffer into Cathepsin E 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 E Substrate Preparation:** For each well, prepare 40 µl of the substrate solution.

39 µl CTSE Assay Buffer
1 µl CTSE Substrate

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

4. **Measurement:** Measure fluorescence (Ex/Em = 320/420 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 (RFU₁ and RFU₂).
5. **Calculations:** Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU₂-RFU₁) values with the time ΔT (T₂-T₁).

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

Note: Irreversible inhibitors that inhibit the Cathepsin E activity completely at the tested concentration will have $\Delta\text{RFU} = 0$ and thus the % Relative Inhibition will be 100%.

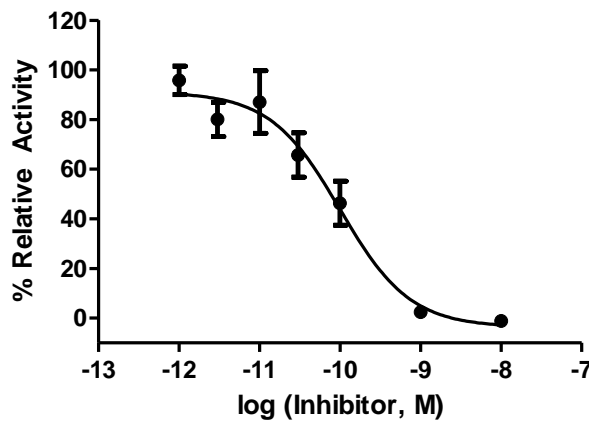


Figure: Inhibition of Cathepsin E activity by CTSE Inhibitor. Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Cathepsin L Activity Fluorometric Assay Kit (K142)	Cathepsin L Antibody (3192)
Cathepsin L (Cleaved) Antibody (3741)	Cathepsin L (Cleaved) Blocking Peptide (3741BP)
Cathepsin L Blocking Peptide (3192BP)	Cathepsin L, human recombinant (1135)
Cathepsin B (1021)	Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin B Antibody (3190)	Cathepsin B Inhibitor Screening Kit (K147)
Cathepsin D (1022)	Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin D Antibody (3191R)	Cathepsin D Blocking Peptide (3191RBP)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)	Cathepsin F Antibody (3371)
Cathepsin F Blocking Peptide (3371BP)	Cathepsin G Activity Assay Kit, Fluorometric (K146)
Cathepsin G Antibody (3370)	Cathepsin G Inhibitor (1982)
Cathepsin G Substrate (2206)	Cathepsin G, human neutrophil (4713)
Cathepsin G Activity Fluorometric Assay Kit (K146)	Cathepsin H (1023)
Cathepsin H Activity Fluorometric Assay Kit (K145)	Cathepsin K Activity Fluorometric Assay Kit (K141)
Procathepsin K, human recombinant (1026)	Procathepsin K, mouse recombinant (1027)
Procathepsin K, rat recombinant (1029)	Cathepsin K Antibody (3588, 3368)
Cathepsin K Blocking Peptide (3588BP, 3368BP)	Human CellExp™ Cathepsin S, human recombinant (7277)
Cathepsin S Activity Fluorometric Assay Kit (K144)	Cathepsin S Inhibitor Screening Kit (K149)
Cathepsin S Antibody (3366, 3366R)	Cathepsin S Blocking Peptide (3366R)

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