Cathepsin E Inhibitor Screening Kit (Fluorometric)

(Catalog #BN00446; 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. Assay Genie'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.

CTSE Substrate-MCA $\xrightarrow{\text{Cathepsin E}}$ Cleaved substrate + MCA (Fluorescence)

CTSE Substrate-MCA $\xrightarrow[\text{+ CTSE Inhibitor}]{\text{Cathepsin E}}$ Decrease in MCA fluorescence/No fluorescence

II. Applications:

- Screening/studying/characterizing inhibitors of Cathepsin E.

III. Kit Contents:

Components	BN00446	Cap Code	Part Number
CTSE Assay Buffer	25 ml	NM	BN00446-1
Human Cathepsin E	1 vial	Green	BN00446-2
CTSE Substrate	0.2 ml	Brown	BN00446-3
CTSE Inhibitor (1 mM Pepstatin A in DMSO)	20 µl	Blue	BN00446-4

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 (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).

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

Copyright © 2019 Reagent Genie Ltd | AssayGenie.com | hello@assaygenie.com

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

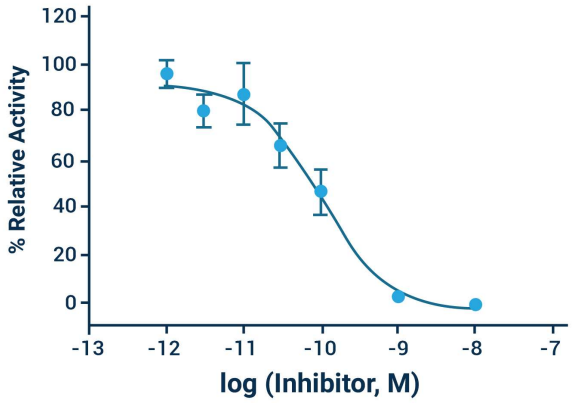

Figure: Inhibition of Cathepsin E activity by CTSE Inhibitor. Assay was performed following the kit protocol.

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

Copyright © 2019 Reagent Genie Ltd | AssayGenie.com | hello@assaygenie.com