Cathepsin S Inhibitor Screening Kit (Fluorometric)

(Catalog #BN00429; 100 assays, Store kit at -20°C)

I. Introduction:

Cathepsin S (CTSS, EC 3.4.22.27) is a lysosomal cysteine proteinase that is suggested to participate in the degradation of antigenic proteins to peptides for presentation on MHC class II molecules. Assay Genie's Cathepsin S Inhibitor Screening Kit utilizes the ability of Cathepsin S 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 S inhibitor, the cleavage of the substrate is reduced/abolished resulting in decrease or total loss of the AFC fluorescence. This high-throughput adaptable assay kit is simple, sensitive, and rapid tool to screen the potential inhibitors of Cathepsin S.

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

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

II. Applications:

- Screening potential inhibitors of Cathepsin S
- Characterize/study Cathepsin S inhibitors

III. Kit Contents:

Components	BN00429	Cap Code	Part Number
CTSS Reaction Buffer	15 ml	WM	BN00429-1
Cathepsin S (human)	1 Vial	Green	BN00429-2
CTSS Substrate, Z-VVR-AFC (10 mM)	0.2 ml	Brown	BN00429-3
CTSS Inhibitor (Z-FF-FMK, 1 mM)	20 µl	Red	BN00429-4

IV. User Supplied Reagents and Equipment:

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

V. Storage & 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:

- **CTSS Reaction Buffer:** Warm CTSS Reaction Buffer to room temperature before use.
- **Cathepsin S (human):** Add 100 µl of CTSS Reaction Buffer to the vial. Aliquot & store at -80°C. Avoid repeated freeze/thaw.

VII. Cathepsin S Inhibitor Screening Protocol:

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

   49 µl CTSS Reaction Buffer
   1 µl diluted Cathepsin S 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 CTSS Reaction Buffer. Add 10 µl diluted test inhibitors (Sample, S) or CTSS Reaction Buffer into Cathepsin S enzyme containing wells (Enzyme Control, EC). For Inhibitor Control (IC), add 1 µl CTSS Inhibitor & 9 µl CTSS Reaction Buffer to Cathepsin S enzyme well(s). Incubate at room temperature for 10-15 min.

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

   38 µl CTSS Reaction Buffer
   2 µl CTSS Substrate

   Mix & add 40 µl of CTSS 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 fluorescence values (RFU1 and RFU2).

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

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

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

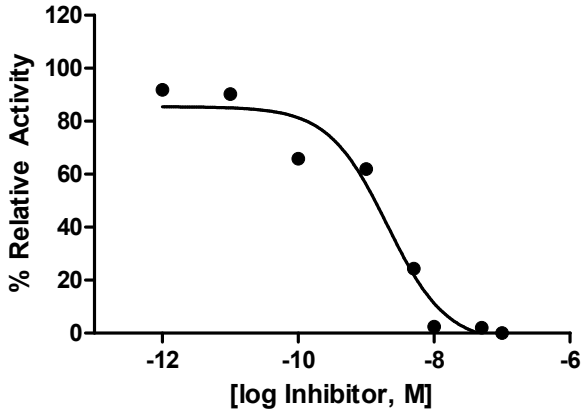

Figure: Inhibition of Cathepsin S activity by CTSS Inhibitor. Assay was performed following kit protocol.

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