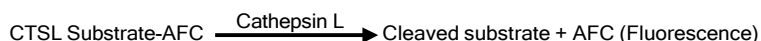


Cathepsin L Inhibitor Screening Kit (Fluorometric)

(Catalog #BN00441; 100 assays, Store kit at -80°C)

I. Introduction:

Cathepsin L (CTSL, EC 3.4.22.15) is a lysosomal cysteine protease that is implicated in protein degradation, arthritis, apoptosis, and cancer. Assay Genie's Cathepsin L Inhibitor Screening Kit utilizes the ability of active Cathepsin L 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 L 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 L.



II. Applications:

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

III. Kit Contents:

Components	BN00441	Cap Code	Part Number
CTSL Assay Buffer	15 ml	WM	BN00441-1
CTSL Reagent	0.1 ml	Clear	BN00441-2
DTT	0.1 ml	Blue	BN00441-3
Cathepsin L (human, 20 mU)	10 µl	Green	BN00441-4
CTSL Substrate, Ac-FR-AFC (10 mM)	0.2 ml	Brown	BN00441-5
CTSL Inhibitor (FF-FMK, 1 mM)	20 µl	Red	BN00441-6

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 -80°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **CTSL Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **CTSL Reagent:** Add 5 µl DTT to CTSL Reagent, mix well, aliquot & store at -20°C. Avoid repeated freeze/thaw.
- **Cathepsin L (human):** Store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **CTSL Substrate (Ac-FR-AFC, 10 mM) and CTSL Inhibitor (FF-FMK, 1 mM):** Bring to RT before use. Store at -20 °C

VI. Cathepsin L Inhibitor Screening Protocol:

1. **Cathepsin L Enzyme Solution Preparation:** Dilute the Cathepsin L Enzyme by adding 1 µl of CTSL Reagent to 1 µl of Cathepsin L

Enzyme as needed. Pre-incubate at RT for 30 min to 1 hr. Further dilute the Cathepsin L Enzyme by adding 8 µl of CTSL Assay Buffer. Gently pipette up & down. For each well, prepare 50 µl of Cathepsin L Enzyme solution as follows:

48 µl CTSL Assay Buffer
1 µl DTT
1 µl diluted Cathepsin L enzyme solution (0.2 mU/µl)

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

Note: Diluted Cathepsin L Enzyme may be stored at -80 °C up to 2 weeks in the presence of 50% glycerol. Long term storage is not recommended. If using diluted Cathepsin L Enzyme solution having 50% glycerol, add 2 µl instead of 1 µl to make the Cathepsin L enzyme solution.

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

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

39 µl CTSL Assay Buffer
1 µl CTSL Substrate

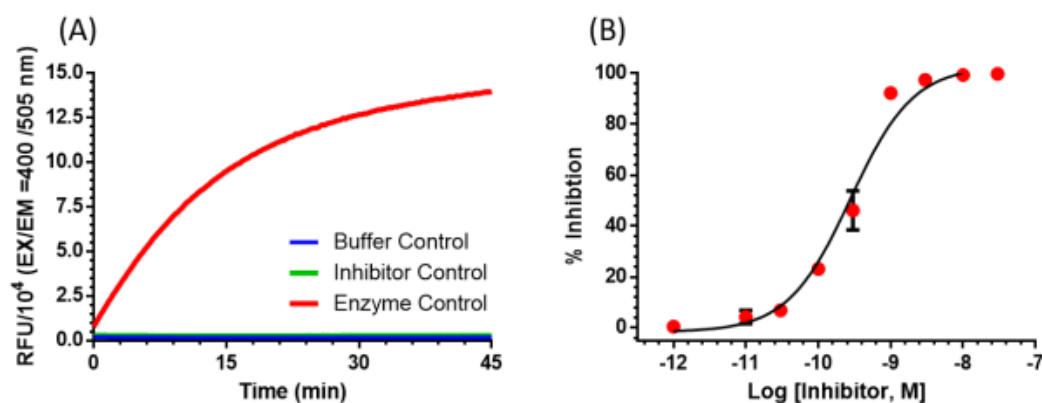
Mix & add 40 µl of Cathepsin L Substrate solution into Enzyme Control, Inhibitor Control & sample wells. Mix well.

4. **Measurement:** Measure the fluorescence in a kinetic mode for 30 min. at 37°C (Ex/Em = 400/505 nm). The graphs is linear up to 15 min. 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 Δ RFU ($\text{RFU}_2 - \text{RFU}_1$) values with the time ΔT ($T_2 - T_1$).

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

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



Figures: (A). Activity of Cathepsin L in the presence and absence of inhibitor (FF-FMK). (B). Inhibition of Cathepsin L activity by CTSL Inhibitor. Assays were performed following the kit protocol.

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