



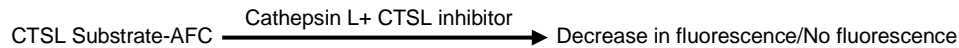
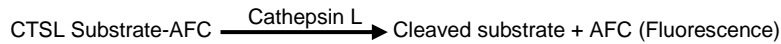
Cathepsin L Inhibitor Screening Kit (Fluorometric)

rev 07/21

(Catalog # K161-100; 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. **BioVision'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. Application:

- Screen/study/characterize potential inhibitors of Cathepsin L

III. Kit Contents:

Components	K161-100	Cap Code	Part Number
CTSL Assay Buffer	15 ml	WM	K161-100-1
CTSL Reagent	0.1 ml	Clear	K161-100-2
DTT	0.1 ml	Blue	K161-100-3
Cathepsin L	10 µl	Green	K161-100-4
CTSL Substrate	0.2 ml	Brown	K161-100-5
CTSL Inhibitor	20 µl	Red	K161-100-6

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 all small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **CTSL Assay Buffer:** Bring to room temperature (RT) before use. Store at 4 °C or -20 °C.
- **CTSL Reagent:** Add 5 µl DTT to CTSL Reagent, mix well. Divide into aliquots & store at -20 °C. Avoid repeated freeze/thaw cycles.
- **Cathepsin L:** Store at -80 °C. Avoid repeated freeze/thaw cycles. 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

Mix well and add 50 µl/well of **Cathepsin L Enzyme solution** into wells of a 96-well white 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 RT for 15 min.

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

39 µl CTSL Assay Buffer
1 µl CTSL Substrate

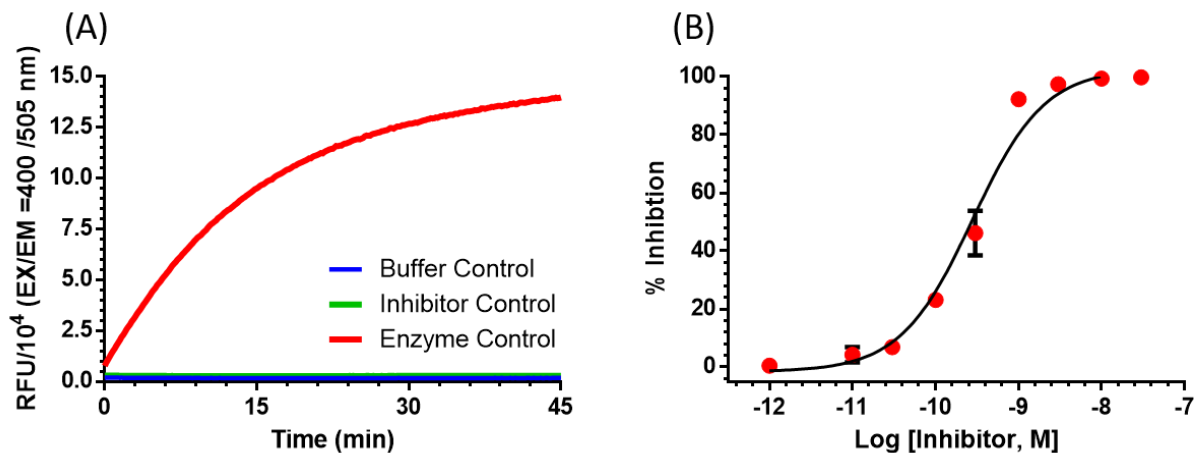
Mix & add 40 µl of Cathepsin L Substrate solution into Enzyme Control, Inhibitor Control & Sample wells. Mix well.

4. **Measurement:** Measure the fluorescence in a kinetic mode for 30 min. at 37 °C (Ex/Em = 400/505 nm). The graph is linear up to 15 min. Choose any 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 ($\text{RFU}_2 - \text{RFU}_1$) 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 $\Delta\text{RFU} = 0$ 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.

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)

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