



Cathepsin K Inhibitor Screening Kit (Fluorometric)

rev 11/20

(Catalog # K150-100; 100 assays, Store kit at -80 °C)

I. Introduction:

Cathepsin K (CTSK, EC 3.4.22.38), a lysosomal cysteine protease, is involved in osteoclastic bone remodeling and resorption. In addition, it also degrades collagen, gelatin and elastin. **Biovision's Cathepsin K Inhibitor Screening Kit** utilizes the ability of active Cathepsin K 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 K-specific 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 K.

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

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

II. Applications:

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

III. Kit Contents:

| Components | K150-100 | Cap Code | Part Number |
|-----------------------------------|----------|----------|-------------|
| CTSK Reaction Buffer | 15 ml | NM | K150-100-1 |
| CTSK Reagent | 100 µl | Blue | K150-100-2 |
| Human Cathepsin K (0.5 mg/ml) | 10 µl | Green | K150-100-3 |
| CTSK Substrate, Ac-LR-AFC (10 mM) | 200 µl | Amber | K150-100-4 |
| CTSK Inhibitor (FF-FMK, 1 mM) | 20 µl | Red | K150-100-5 |

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 -80 °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:

- **CTSK Reaction Buffer:** Warm to room temperature (RT) before use.
- **CTSK Reagent:** Divide into aliquots & store at -20 °C. Avoid repeated freeze/thaw cycles.
- **Human Cathepsin K:** Add 100 µl of CTSK Reaction Buffer. Gently pipette up & down to dissolve completely. Divide into aliquots & store at -80 °C. Avoid freeze/thaw cycles.
- **CTSK Substrate, Ac-LR-AFC (10 mM):** Protect CTSK Substrate from light.

VII. Cathepsin K Inhibitor Screening Protocol:

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

48 µl CTSK Reaction Buffer
1 µl CTSK Reagent
1 µl Cathepsin K 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 CTSK Reaction Buffer. Add 10 µl diluted test inhibitors (Sample, S) or CTSK Reaction Buffer into Cathepsin K enzyme containing wells (Enzyme Control, EC). For Inhibitor Control (IC), add 1 µl CTSK Inhibitor and 9 µl CTSK Reaction Buffer into Cathepsin K enzyme well(s). Incubate at RT for 10-15 min.

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

38 µl CTSK Reaction Buffer
2 µl CTSK Substrate

Mix & add 40 µl of Cathepsin K 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 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 ($T_2 - T_1$).

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



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

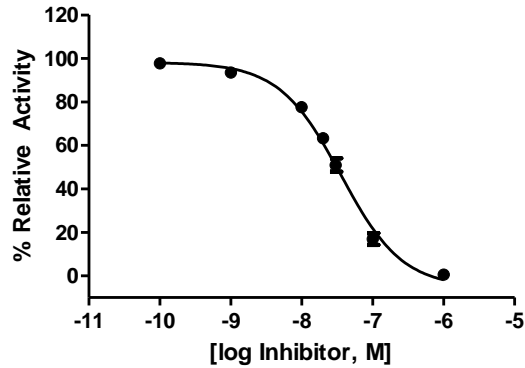


Figure: Inhibition of Cathepsin K activity by CTSK Inhibitor. Assay was performed following kit protocol.

VIII. RELATED PRODUCTS:

- | | |
|---|---|
| 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) | Cathepsin L Activity Fluorometric Assay Kit (K142) |
| Cathepsin S Activity Fluorometric Assay Kit (K144) | Cathepsin S Inhibitor Screening Kit (K149) |
| Cathepsin S Antibody (3366, 3366R) | Cathepsin S Blocking Peptide (3366R) |
| Human CellExp™ Cathepsin S, human recombinant (7277) | |

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