ab197012
Cathepsin L Inhibitor Screening Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate screening of inhibitors of Cathepsin L.

This product is for research use only and is not intended for diagnostic use.

Version 3a Last Updated 2 June 2025

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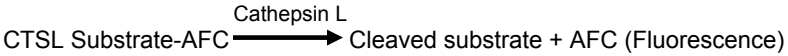
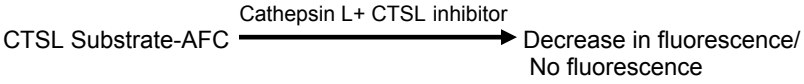
1. BACKGROUND

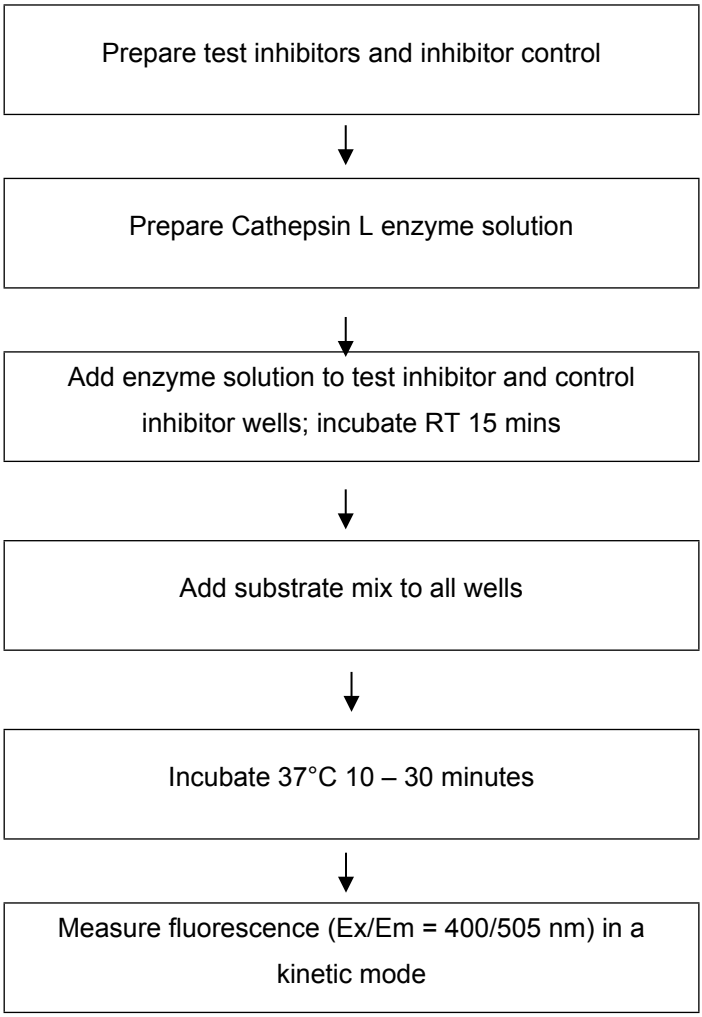
Cathepsin L Inhibitor Screening Kit (fluorometric) (ab197012) uses the ability of active Cathepsin L to cleave the synthetic AFC-based peptide substrate to release AFC, which can be easily quantified using a fluorometer or fluorescence microplate reader. In the presence of a Cathepsin L inhibitor, the 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.





Cathepsin L (CTSL) is a lysosomal cysteine protease that is implicated in protein degradation, arthritis, apoptosis, and cancer.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Cathepsin L Assay Buffer	15 mL	-20°C	-20°C
Cathepsin L Activator	0.1 mL	-20°C	-20°C
DTT II	100 µL	-20°C	-20°C
Human Cathepsin L	10 µL	-20°C	-20°C
Cathepsin L Substrate	200 µL	-20°C	-20°C
Cathepsin Inhibitor	20 µL	-20°C	-20°C

PLEASE NOTE: Cathepsin Inhibitor was previously labelled as CTSL Inhibitor (FF-FMK) (1 mM), and Cathepsin L Assay Buffer as CTSL Assay Buffer, and Cathepsin L Activator as CTSL Reagent, and

Cathepsin L Substrate as CTSL Substrate, Ac-FR-AFC (10 mM), and DTT II as DTT. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 400/505 nm
- 96 well plate: white plate
- Heat block or water bath

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Cathepsin L Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 **Cathepsin L Activator:**

Add 5 µL of DTT II to Cathepsin L Activator. Mix well. Keep on ice while in use. Aliquot CTSL regent so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C.

9.3 **DDT:**

Ready to use as supplied. Keep on ice while in use. Store at -20°C

9.4 **Human Cathepsin L:**

Ready to use as supplied. Keep on ice while in use. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.5 **Cathepsin L Substrate:**

Ready to use as supplied. Keep on ice while in use. Aliquot Cathepsin L Substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.6 **Cathepsin Inhibitor:**

Ready to use as supplied. Keep on ice while in use. Aliquot Cathepsin Inhibitor so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. SAMPLE PREPARATION

- Always prepare a fresh set of samples and controls for every use.

10.1 Screening Inhibitor Compounds:

10.1.1 Dissolve test compounds into appropriate solvent.

10.1.2 Dilute to 10X the desired test concentration with Cathepsin L Assay Buffer.

NOTE: *We suggest using different volumes of testing compounds if effective concentration is unknown.*

10.2 Inhibitor Control:

10.2.1 Dilute Cathepsin Inhibitor 1:10 in Cathepsin L Assay Buffer (initial recommendation= 1 µL Cathepsin Inhibitor + 9 µL Cathepsin L Assay Buffer).

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**
- **Diluted Cathepsin L Enzyme solution 0.2 mU/µL (Section 11.1) may be stored at -80°C up to 2 weeks in the presence of 50% glycerol. Long term storage is not recommended.**

11.1 Cathepsin L Enzyme Solution preparation:

- Add 1 µL Cathepsin L Activator to 1µL Cathepsin L Enzyme (solution = 1mU/µL). Prepare as much solution as needed.
- Pre-incubate at room temperature for 30 – 60 minutes.
- Dilute Cathepsin L to 0.2 mU/µL by adding 8 µL of Cathepsin L Assay Buffer/CTSL Assay buffer to 2 µL 1mU/µL Enzyme solution.
- Gently pipette up and down.
- For each well, prepare 50 µL of Cathepsin L Enzyme Solution as follows:

Component	Reaction Mix (µL)
Cathepsin L Assay Buffer	**48**
DTT II	**1**
0.2 mU/µL Cathepsin L Enzyme solution*	**1**

**If using diluted Cathepsin L Enzyme solution (0.2 mU/µL) containng 50% glycerol, add 2 µL instead of 1 µL to make the Cathepsin L enzyme solution.*

Mix enough reagents for the number of tests (samples, inhibitor controls and enzyme control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency.

11.2 **Set up Reaction wells:**

Component	Sample (S) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Diluted Cathepsin L Enzyme Solution	**50**	**50**	**50**
Test inhibitor	**10**	**0**	**0**
Cathepsin L Assay Buffer	**0**	**10**	**0**
Diluted Cathepsin Inhibitor			**10**

- OPTIONAL: Solvent control (BC) = 10 µL solvent. NOTE: preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2%, include solvent control to test the effect on the solvent on enzyme activity.

11.3 Incubate at room temperature for 15 minutes.

11.4 **Cathepsin Substrate Mix:**

Prepare 40 µL of Substrate Mix for each reaction

Component	Colorimetric Reaction Mix (µL)
Cathepsin L Assay Buffer	**39**
Cathepsin L Substrate	**1**

Mix enough reagents for the number of assays (sample, enzyme control and inhibitory control wells) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards +1).

11.5 Add 40 µL of Substrate Mix into each sample (S), enzyme control (EC) and inhibitor control (IC) wells.

11.6 Mix well and incubate at 37°C for 30 min protected from light.

11.7 Measure the fluorescence in a kinetic mode, every 2 – 3 minutes, at 37°C for 30 minutes at Ex/Em = 400/505 nm. The graph is linear up to 15 minutes. Choose two time points (T1 and

T2) in the linear range of the plot to obtain the corresponding values for fluorescence (RFU1 and RFU2).

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Average the duplicate reading for each sample, Enzyme Control (EC) and Inhibitor Control.

12.2 Subtract the mean absorbance value of the Solvent Control (BC) if necessary from all controls and sample readings. This is the corrected absorbance.

12.3 Plot the corrected absorbance values for each sample and control as a function of the final concentration of compound.

12.4 Calculate the slope for all samples (S), Inhibition Control and Enzyme Control (EC):

$$Slope = \left(\frac{RFU2 - RFU1}{T2 - T1}\right) * D$$

Where:

RFU1 = Fluorescence value at Ex/Em = 400/505 nm at Time T_1.

RFU2 = Fluorescence value at Ex/Em = 400/505 nm at Time T_2.

T1 = Time of the first reading (A_1) in minutes.

T2 = Time of the second reading (A_2) in minutes.

D = sample dilution factor.

12.5 Calculate the % Relative inhibitions as follows

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

NOTE: *Irreversible inhibitors that inhibit the Cathepsin L activity completely at the tested concentration will have ΔRFU = 0 and thus the % relative inhibition will be 100%.*

13. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

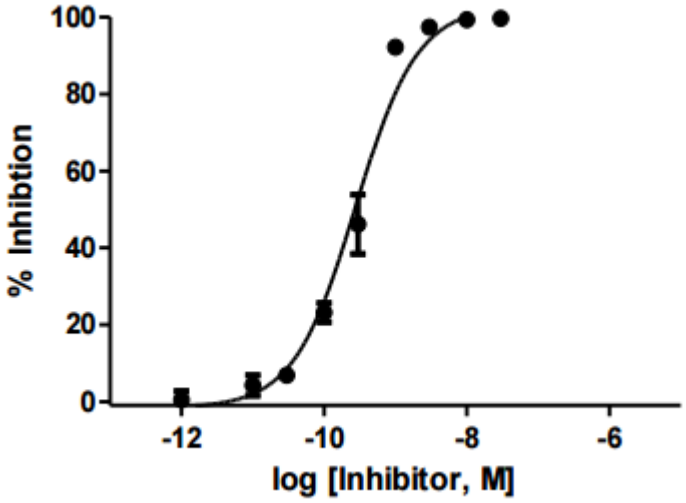


Figure 1. Inhibition of Cathepsin L activity by CTSL Inhibitor.

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare Cathepsin L Assay Buffer/assay buffer, Cathepsin L Activator, DTT II, Cathepsin L, Cathepsin L Substrate and inhibitor (aliquot if necessary); get equipment ready.
- Prepare test inhibitors and Cathepsin Inhibitor.
- Prepare diluted Cathepsin L Enzyme Solution.
- Set up plate as follows for samples, enzyme control and inhibitor controls:

Component	Sample (S) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Diluted Cathepsin L Enzyme Solution	**50**	**50**	**50**
Test inhibitor	**10**	**0**	**0**
Cathepsin L Assay Buffer	**0**	**10**	**0**
Diluted Cathepsin Inhibitor	**0**	**0**	**10**

- Prepare 40 µL Cathepsin Substrate Mix per well (39 µL Cathepsin L Assay Buffer + 1 µL Cathepsin L Substrate); add 40 µL Substrate Mix to each S, EC and IC well.
- Measure plate at 37°C at Ex/Em= 400/505 nm for fluorometric assay in a kinetic mode for 30 minutes, protected from light.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

Technical Support

For all technical or commercial enquiries please go to:

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