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ab211080 Cathepsin H Inhibitor Screening Kit (Fluorometric)

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

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

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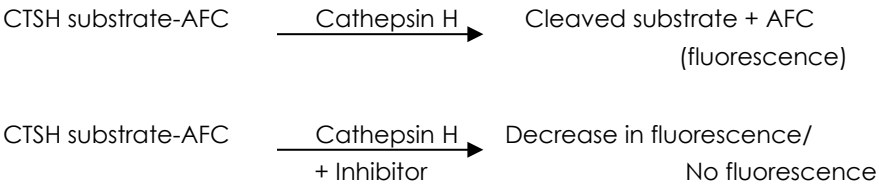
1. Overview

Cathepsin H Inhibitor Screening Kit (Fluorometric) (ab211080) provides a rapid, simple, sensitive and reliable test suitable for high-throughput screening of Cathepsin H inhibitors. It utilizes the ability of an active Cathepsin H to cleave a synthetic AFC-based peptide substrate to release free AFC, which can be easily quantified using a fluorometer or fluorescence microplate reader. In the presence of a Cathepsin H-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 can be used to screen/study/characterize potential inhibitors of Cathepsin H.



Cathepsin H (CTSH, EC 3.4.22.16), a lysosomal cysteine protease, is important in the overall degradation of lysosomal proteins. It is composed of a dimer of disulfide-linked heavy and light chains, both produced from a single protein precursor. The encoded protein, which belongs to the peptidase C1 protein family, can act both as an aminopeptidase and as an endopeptidase. Increased expression of this gene has been correlated with malignant progression of prostate tumors.

2. Protocol Summary

Screening compound & controls preparation

Enzyme and substrate solution preparation

Add enzyme solution to wells.
Incubate for 15 minutes at RT

Add Substrate Solution to wells

Measure fluorescence at Ex/Em (400/505 nm) in kinetic mode
for 1 – 2 hours at 37°C

**For kinetic mode detection, incubation time given in this summary is for guidance only*

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C 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 the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (Before prep)	Storage temperature (After prep)
CTSH Assay Buffer	25 mL	-20°C	4°C / -20°C
CTSH Reagent	100 µL	-20°C	-20°C
Human Cathepsin H (5 µg)	1 vial	-20°C	-20°C
CTSH Substrate (in DMSO)	200 µL	-20°C	-20°C
CTSH Inhibitor (1 mM in DMSO)	20 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 400/505 nm
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably white

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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, controls and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 CTSH Assay Buffer (25 mL):

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

9.2 CTSH Substrate (200 µL in DMSO):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.3 CTSH Reagent (100 µL):

Ready to use as supplied. Aliquot reagent so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeat freeze/thaw.

9.4 Human Cathepsin H (lyophilized, 5 µg):

Reconstitute Cathepsin H in 110 µL of CTSH Assay Buffer. Keep on ice while in use. Aliquot reconstituted enzyme so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within 2 months.

9.5 CTSH Inhibitor (1 mM in DMSO) (20 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot inhibitor so that you have enough volume to perform the desired number of assay. Store at -20°C. Avoid repeat freeze/thaw.

10. Sample Preparation

General sample information:

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

10.1 Screening Compounds:

10.1.1 Dissolve test inhibitors into proper solvent.

10.1.2 Dilute to 10X the desired test concentration with CTSH Assay Buffer before use.

Δ Note: We suggest using different concentrations of test compounds if effective concentration is unknown.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

Δ Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well with the same final concentration of the solvent as in the inhibitor sample as solvent control.

11.1 Set up Reaction wells:

- Screening samples compound wells (S) = 10 µL test compounds.
- Inhibitor Control wells (IC) = 1 µL CTSH Inhibitor + 9 µL CTSH Assay Buffer.
- Enzyme Control wells (EC) = 10 µL CTSH Assay Buffer.
- OPTIONAL: Solvent control (SC) = 10 µL solvent.

11.2 Prepare Cathepsin H Enzyme Solution:

11.2.1 Prepare 50 µL of Enzyme Solution for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	CTSH Enzyme Mix (µL)
CTSH Assay Buffer	48
Reconstituted Human Cathepsin H	1
CTSH Reagent	1

11.2.2 Mix well and add to each well.

11.2.3 Incubate plate at room temperature for 15 minutes.

11.3 Cathepsin H Substrate Preparation:

11.3.1 Prepare 40 µL of Substrate Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	CTSH Substrate Mix (µL)
CTSH Assay Buffer	39
CTSH Substrate	1

11.3.2 Add 40 µL of Substrate Mix into each well. Mix well.

11.4 Measurement:

11.4.1 Measure fluorescence (Ex/Em = 400/505 nm) in a kinetic mode for 1 - 2 hours at 37°C. Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

12. Calculations

– Use only the linear rate for calculation.

12.1 Average the duplicate reading for each sample test compound (S), inhibitor control (IC) and enzyme control (EC).

12.2 Plot standard curve readings and draw the line of the best fit to construct the curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

12.3 Choose two points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

12.4 Calculate Slope (ΔRFU/ΔT) for all samples (S), Enzyme Control (EC) and Inhibitor control (IC), if desired, as follows:

$$\Delta RFU/\Delta T = (RFU2 - RFU1)/ (T2 - T1)$$

12.5 Calculate the % Relative Inhibitions as follows:

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

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

Δ Note: If RFU of SC < RFU of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent if possible.

If RFU of S < RFU of EC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. Typical Data

Data provided **for demonstration purposes only.**

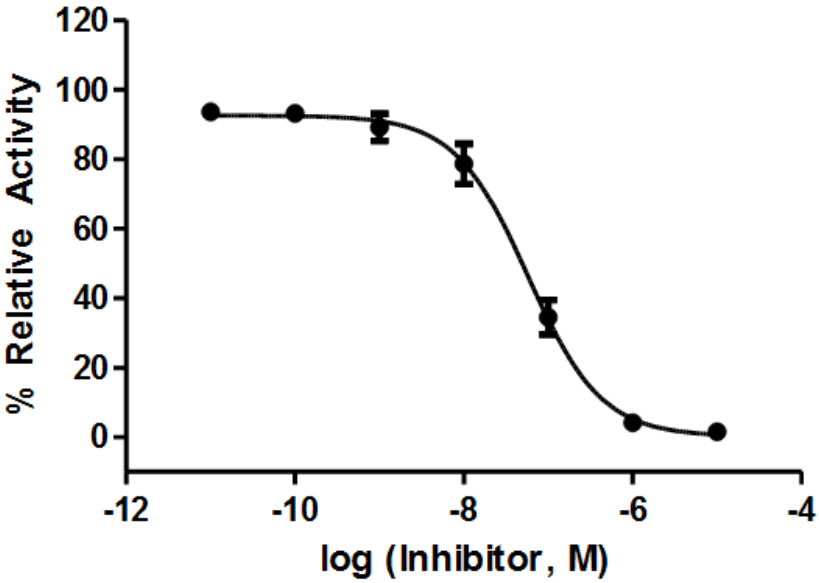


Figure 1. Typical inhibition curve of Cathepsin H by Cathepsin H inhibitor.

14. Quick Assay Procedure

Δ 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 components and aliquot if necessary; get equipment ready.
- Prepare test compounds in suitable solvents; dilute if appropriate.
- Prepare Cathepsin H Enzyme Solution (50 µL/well) by adding 2 µL of human Cathepsin H to 48 µL of CTSH Assay Buffer. Prepare a mix for all wells.
- Prepare Cathepsin H Substrate mix (40 µL/well) by adding 1 µL of CTSH Substrate to 39 µL of CTSH Assay Buffer. Prepare a mix for all wells.
- Set up plate as follows:

Component	Sample (S) (µL)	Solvent control (SC) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Test Compound	10	0	0	0
CTSH inhibitor control	0	0	0	1
Solvent test compound	0	10	0	0
Assay Buffer	0	0	10	9
Cathepsin H Enzyme Solution	50	50	50	50
Incubate 15 minutes at RT				
Add 40 µL Cathepsin H Substrate Mix				

- Measure plate at Ex/Em= 400/505 nm in kinetic mode for 1-2 hours at 37°C.

15. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Assay with erratic readings	Pipetting errors	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
No fluorescence above background in inhibitor wells	Inhibitor concentration is too high	Reduce concentration of inhibitor and re-do assay
No inhibition seen in test compound wells	Inhibitor concentration is not high enough	Increase concentration of inhibitor and re-do assay
	Compound is not an inhibitor	Use another compound for your test

16. Notes

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