ab185438

Cathepsin B Inhibitor Screening Kit (Fluorometric)

Instructions for Use

For the sensitive and accurate screening of Cathepsin B inhibitors in a variety of samples

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version: 5 Last Updated: 7 February 2024

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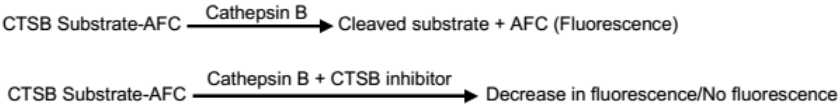
1. Overview

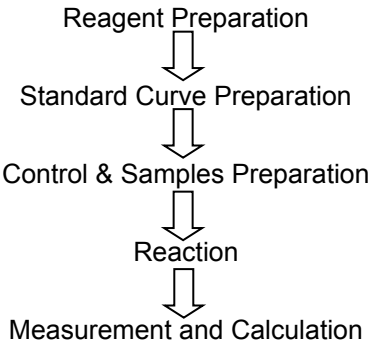
Cathepsin B (CTSB, EC 3.4.22.1) is a lysosomal cysteine proteinase that is suggested to participate in intracellular degradation and turnover of proteins. It has also been implicated in tumor invasion and metastasis.

Abcam's Cathepsin B Inhibitor Screening Kit (ab185438) utilizes the ability of Cathepsin B 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 B-specific inhibitor, the cleavage of the substrate is reduced/abolished resulting in decrease or total loss of the AFC fluorescence. This high-throughput adaptable assay kit is simple, sensitive, and rapid tool to screen the potential inhibitors of Cathepsin B.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
Cathepsin B Assay Buffer/CTSB Reaction Buffer	15 mL
DTT I/CTSB Reagent	150 µL
Human Cathepsin B/Cathepsin B	5 µL
Cathepsin B Substrate/CTSB Substrate, Ac-RR-AFC (10 mM)	0.2 mL
Cathepsin Inhibitor/CTSB Inhibitor (F-F-FMK, 1 mM)	20 µL

4. Storage and Stability

Upon arrival, store kit at -20°C and protect from light.

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

5. Materials Required, Not Supplied

- 96-well **white** plate with flat bottom (for this specific assay, white plates are preferred to black plates)
- Multi-well spectrophotometer (ELISA reader)
- Multi-channel pipette
- Distilled water

6. Reagent Preparation

1. **Cathepsin B Assay Buffer/CTSB Reaction Buffer:**
   Ready to use as supplied. Warm Cathepsin B Assay Buffer/CTSB Reaction Buffer to room temperature before use.

2. **DTT I/CTSB Reagent**
   Ready to use as supplied. Aliquot & store at -20°C. Avoid repeated freeze/thaw.

3. **Cathepsin B Substrate/CTSB Substrate (Ac-RR-AFC):**
   Ready to use as supplied. Warm Cathepsin B Substrate/CTSB Substrate to room temperature before use.

4. **Human Cathepsin B/Cathepsin B:**
   Add 105 µl of Cathepsin B Assay Buffer/CTSB Reaction Buffer to the vial. Gently pipette up & down to dissolve completely. Aliquot & store at -80°C. Avoid repeated freeze/thaw.

5. **Cathepsin Inhibitor/CTSB Inhibitor (F-F-FMK):**
   Ready to use as supplied. Store at -20°C.

7. Inhibitor Screening Protocol

1. Cathepsin B Enzyme Solution Preparation:

a) For each well, prepare 50 µL of Cathepsin B Enzyme solution containing:

	Enzyme Solution Preparation
Cathepsin B Assay Buffer/CTSB Reaction Buffer	48 µL x (Nb samples + Controls + 1)
DTT I/CTSB Reagent	1 µL x (Nb samples + Controls + 1)
Human Cathepsin B/Cathepsin B	1 µL x (Nb samples + Controls + 1)

Mix enough reagents for the number of assays (samples, inhibitor control and blank control) to be performed. We recommend preparing a Master Mix Enzyme Solution as stated above to ensure consistency.

2. Sample Preparation

a) Screening Compounds:

Dissolve test inhibitors into the appropriate solvent in order to prepare a 10x stock solution of test inhibitor using Cathepsin B Assay Buffer/CTSB Reaction Buffer. Add 10 µl diluted test inhibitors (Sample, S) or Cathepsin B Assay Buffer/CTSB Reaction Buffer into Cathepsin B enzyme containing wells (Enzyme Control, EC).

b) Inhibitor Control (IC):

Add 1 µl Cathepsin Inhibitor/CTSB Inhibitor & 9 µl CTSB Reaction

Buffer into Cathepsin B enzyme well(s).

c) Incubate plate at room temperature for 10 – 15 min.

3. Cathepsin B Substrate Preparation:

a) For each well, prepare 40 µL of Cathepsin B Substrate solution containing:

	Substrate Solution Preparation
Cathepsin B Assay Buffer/CTSB Reaction Buffer	38 µL x (Nb samples + Controls + 1)
Cathepsin B Substrate/CTSB Substrate	2 µL x (Nb samples + Controls + 1)

Mix enough reagents for the number of assays (samples, inhibitor control and blank control) to be performed. We recommend preparing a Master Mix Substrate Solution as stated above to ensure consistency.

b) Add 40 µL of Cathepsin B Substrate solution into each well. Mix well.

4. Measurement:

Measure the fluorescence at Ex/Em = 400/ 505 nm in a kinetic mode for 30-60 min at 37°C. Choose two time points ($\mathbf{T_1}$ & $\mathbf{T_2}$) in the linear range of the plot and obtain the corresponding values for the fluorescence ($\mathbf{RFU_1}$ and $\mathbf{RFU_2}$).

NOTE: It is essential to read RFU1 and RFU2 in the reaction linear range as calculations will be more accurate.

8. Data Analysis

Calculate the slope for all test Inhibitor Samples [S] and Enzyme Control (EC) by dividing the net ΔRFU (RFU2-RFU1) values with the time ΔT (T2-T1).

NOTE: if reading of Black control is high, subtract from all the readings.

Calculate the relative inhibition as follows:

$$\%\ Relative\ Inhibition = \frac{Slope\ of\ EC - Slope\ of\ Sample}{Slope\ of\ EC} X\ 100$$

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

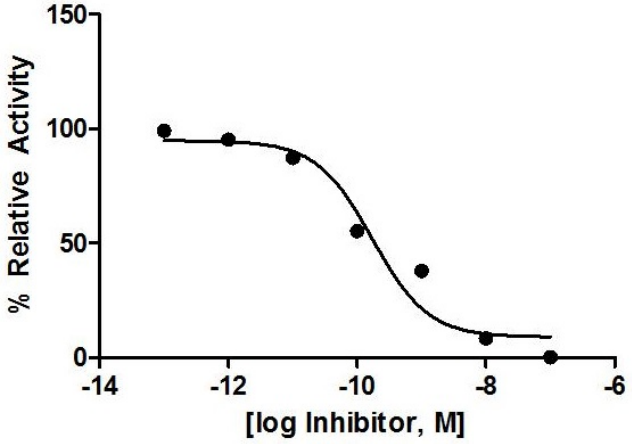


Figure 2. : Inhibition of Cathepsin B activity by Cathepsin B Inhibitor Screening Kit was performed following kit protocol.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

For all technical and commercial enquires please go to:

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