ab185437

Cathepsin S Inhibitor Screening Kit (Fluorometric)

Instructions for Use

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

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

Version: 3 Last Updated: 12 April 2018

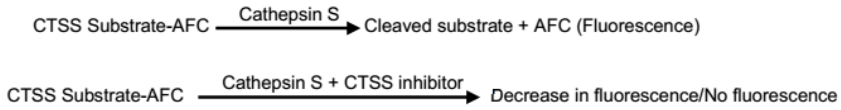
Table of Contents

1. Overview

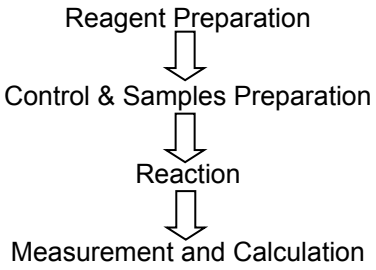
Cathepsin S (CTSS, EC 3.4.22.27) is a lysosomal cysteine proteinase that is suggested to participate in the degradation of antigenic proteins to peptides for presentation on MHC class II molecules

Abcam's Cathepsin S Inhibitor Screening Kit (ab185437) utilizes the ability of Cathepsin S 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 S 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 S.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
CTSS Reaction Buffer	15 mL
Cathepsin S	1 vial
CTSS Substrate, Z-VVR-AFC (10 mM)	0.2 mL
CTSS Inhibitor (Z-FF-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. **CTSS Reaction Buffer:**
   Ready to use as supplied. Warm CTSS Reaction Buffer to room temperature before use.

2. **CTSS Substrate (Z-VVR-AFC):**
   Ready to use as supplied. Warm CTSS Reaction Buffer to room temperature before use.

3. **Cathepsin S:**
   Add 100 µl of CTSS Reaction Buffer to the vial. Aliquot and store at -80°C. Avoid repeated freeze/thaw.

4. **CTSS Inhibitor (Z-FF-FMK):**
   Ready to use as supplied. Store at -20°C.

7. Inhibitor Screening Protocol

1. **Sample Preparation**

   Cathepsin S Enzyme Solution Preparation:

   For each well, prepare 50 µL of Cathepsin S Enzyme solution containing:

	Enzyme Solution Preparation
CTSS Reaction Buffer	49 µL x (Nb samples + Controls + 1)
Cathepsin S	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 CTSS Reaction buffer. Add 10 µl diluted test inhibitors or CTSS Reaction Buffer into Cathepsin S enzyme containing wells (Enzyme Control,).

b) **Inhibitor Control (IC):**

   Add 1 µl CTSS Inhibitor & 9 µl CTSS Reaction Buffer to Cathepsin S enzyme well(s).

Incubate at room temperature for 10-15 minutes.

3. **Cathepsin S Substrate Preparation:**

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

	Substrate Solution Preparation
CTSS Reaction Buffer	38 µL x (Nb samples + Controls + 1)
CTSS 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 S 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. 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**).

NOTE: It is essential to read RFU_1 and RFU_2 in the reaction linear range as calculations will be more accurate.

8. Data Analysis

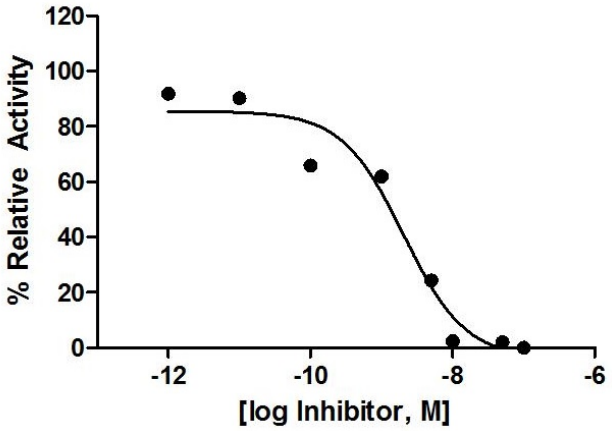
Calculate the slope for all test Inhibitor Samples [S] and Enzyme Control (EC) by dividing the net ΔRFU (RFU2-RFU1) values with the time ΔT (T2-T1).

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 S activity completely at the tested concentration will have ΔRFU = 0 and thus the % Relative Inhibition will be 100%.



Inhibition of Cathepsin S activity by Cathepsin S 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)

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).

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp