ab126779

Cathepsin D Inhibitor Screening Kit

Instructions for Use

For the rapid, sensitive and accurate detection of Cathepsin D inhibition by various compounds

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

Version v9a, Last updated 9 July 2025

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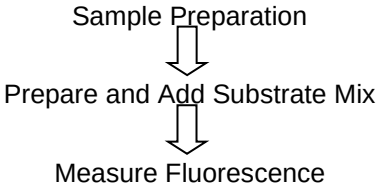
1. Overview

Apoptosis can be mediated by mechanisms other than the traditional caspase-mediated cleavage cascade. There is growing recognition that alternative proteolytic enzymes such as the lysosomal cathepsin proteases may initiate or propagate proapoptotic signals. Cathepsins are lysosomal enzymes that are also used as sensitive markers in various toxicological investigations.

Abcam's Cathepsin D Inhibitior Screening Kit is a fluorescence-based assay that utilizes the preferred cathepsin D substrate sequence GKPILFFRLK(Dnp)-D-R-NH2) labeled with MCA. Cathepsin D will cleave the synthetic substrate to release the quenched fluorescent group MCA, which can then easily be measured using a fluorometer or fluorescence plate reader at Ex/Em = 328/460 nm. The relative efficacy of test inhibitors are compared to the positive control inhibitor, Pepstatin A (IC_{50} < 0.1 nM). The Cathepsin D assay is simple, straightforward, and can be adapted to 96-well plate assays and is suitable for high throughput screening (HTS).

2. Protocol Summary



3. Components and Storage

All components in this kit are shipped on blue ice and are suitable for storage at -80ºC, unless reconstituted. Upon receipt, immediately store kit at -80ºC in the dark. Individual components may be stored at alternative temperatures as show in the table below.

A. Kit Components

Item	Quantity	Storage Temperature
CD Reaction Buffer	10 mL	–80°C or +4°C
Substrate II	0.2 mL	–80°C or –20°C
Human Cathepsin D	1 vial	–80°C
Pepstatin A Solution	20 µL	–80°C or –20°C

- All reagents are stable for 6 months under proper storage conditions.
- Store CD Reaction Buffer at +4°C after opening

HUMAN CATHEPSIN D: Reconstitute with 500 µL of dH_2O, aliquot and store at -80 °C. Avoid freeze/thaw cycles.

PEPSTATIN A SOLUTION: To make working solution, take 2 µl of Pepstatin A Solution stock solution and dilute with 798 µl CD Reaction

Buffer. Working solution is stable for a day at room temperature, after which it should be discarded.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorometer or fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Prepare **Positive Control** by mixing 5 µl reconstituted Human Cathepsin D with 45 µl CD Reaction Buffer.

2. Prepare a **Background Control** with 50 µl of CD Reaction Buffer alone.

3. Prepare an **Inhibitor Reference Control** by mixing 5 µl reconstituted Human Cathepsin D with 10 µl of the diluted Pepstatin A Solution and 35 µl CD Reaction Buffer.

4. Prepare **Test Inhibitor Samples** by mixing 5 µl reconstituted Human Cathepsin D with 10 µl of the Test Inhibitor and 35 µl CD Reaction Buffer.

5. Pre-incubate Controls and Test Samples at 37 °C for 10 min.

6. Prepare Substrate Mix:

Substrate II	2 µl
Reaction Buffer	48 µl

   Add 50 µl to each well containing Controls and Test Samples. Tap plate gently to mix then incubate at 37°C for 1-2 hour.

7. Read samples in a fluorometer equipped with a 320-nm excitation filter and 420-nm emission filter.

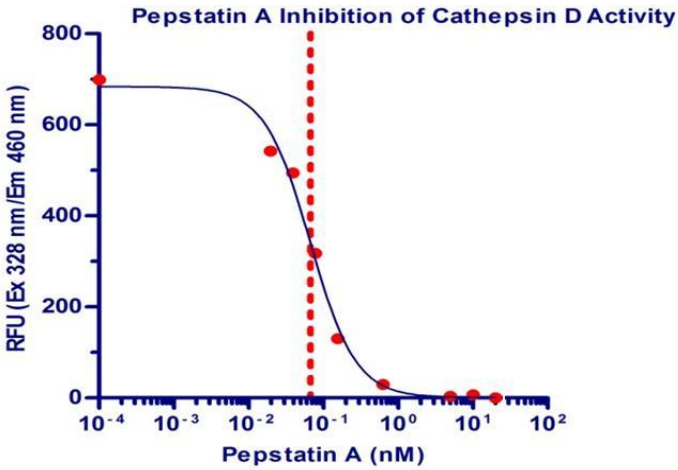
5. Data Analysis

Calculation:

$$\% \text{ Inhibiton} = \left(1 - \frac{(\text{RFU Test Inhibitor} - \text{RFU Bkgd Control})}{(\text{RFU Positive Control} - \text{RFU Bkgd Control})}\right) \times 100\%$$

Or

$$\% \text{ Inhibiton Relative to Pepstatin A} = \left(1 - \frac{(\text{RFU Test Sample} - \text{RFU Bkgd Control})}{(\text{RFU Negative Control} - \text{RFU Bkgd Control})}\right) \times 100\%$$



Typical Pepstatin A Inhibition Profile of Human Cathepsin D Activity. Red line denotes an IC_{50} value of 0.067 nM. Results were analyzed by fluorescence plate reader according to the kit instructions.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Cells did not lyse completely	Re-suspend the cell pellet in the lysis buffer and incubate as described in the datasheet
	Experiment was not performed at optimal time after apoptosis induction	Perform a time-course induction experiment for apoptosis
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
High Background	Increased amount of cell lysate used	Refer to datasheet and use the suggested cell number to prepare lysates
	Increased amounts of components added due to incorrect pipetting	Use calibrated pipettes
	Incubation of cell samples for extended periods	Refer to datasheet and incubate for exact times
	Use of expired kit or improperly stored reagents	Always check the expiry date and store the individual components appropriately
	Contaminated cells	Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	Cells did not initiate apoptosis	Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	Very few cells used for analysis	Refer to datasheet for appropriate cell number

Lower signal levels	Use of samples stored for a long time	Use fresh samples or aliquot and store and use within one month for the assay
	Incorrect setting of the equipment used to read samples	Refer to datasheet and use the recommended filter setting
	Allowing the reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
Unanticipated results	Measured at incorrect wavelength	Check the equipment and the filter setting
	Cell samples contain interfering substances	Troubleshoot if it interferes with the kit (run proper controls)
Samples with erratic readings	Uneven number of cells seeded in the wells	Seed only equal number of healthy cells (correct passage number)
	Samples prepared in a different buffer	Use the cell lysis buffer provided in the kit
	Adherent cells dislodged and lost at the time of experiment	Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters
	Cell/ tissue samples were not completely homogenized	Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples, if needed to use multiple times
	Presence of interfering substance in the sample	Troubleshoot as needed
	Use of old or inappropriately stored samples	Use fresh samples or store at correct temperatures until use

Problem	Reason	Solution
General issues	Improperly thawed components	Thaw all components completely and mix gently before use
	Incorrect incubation times or temperatures	Refer to datasheet & verify the correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Air bubbles formed in the well/tube	Pipette gently against the wall of the well/tubes
	Substituting reagents from older kits/ lots	Use fresh components from the same kit
	Use of a different 96-well plate	Fluorescence: Black plates; Absorbance: Clear plates

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).

Technical Support

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