ab65305

Cathepsin H Activity Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Cathepsin H activity in various samples.

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

Table of Contents

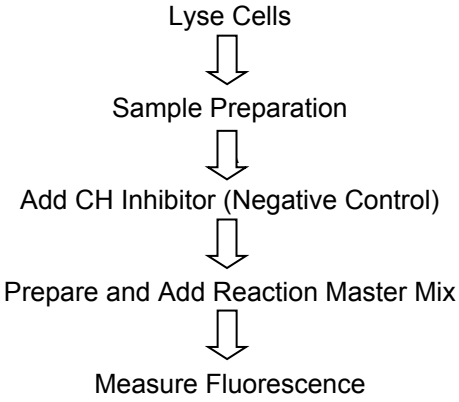
1. Overview

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 pro-apoptotic signals. Cathepsins are lysosomal enzymes that are also used as sensitive markers in various toxicological investigations.

Abcam's Cathepsin H Activity Assay Kit is a fluorescence-based assay that utilizes the preferred Cathepsin H (CH) substrate Arginine labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain Cathepsin H will cleave the synthetic substrate R-AFC to release free AFC. The released AFC can easily be quantified using a fluorometer or fluorescence plate reader.

The Cathepsin H assay is simple, straightforward, and can be adapted to 96-well plate assays. Assay conditions have been optimized to obtain the maximal activity.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
CH Cell Lysis Buffer	25 mL
CH Reaction Buffer	5 mL
CH Substrate R-AFC (10 mM)	200 µL
CH Inhibitor (1mM)	20 µL

* Store kit at -20°C (Store CH Cell Lysis Buffer and CH Reaction Buffer at +4°C after opening). Protect CH Substrate from light. All reagents are stable for 6 months under proper storage conditions.

B. Additional Materials Required

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

4. Assay Protocol

1. Collect cells (10^6) by centrifugation. If the sample is tissue, use 10 mg tissue. Lyse cells or tissue in 50 µl of chilled CH Cell Lysis Buffer. Incubate cells on ice for 10 minutes. Vortex for 5 minutes.

2. Centrifuge 13000 rpm for 5 min in bench-top micro-centrifuge to remove insoluble materials. Transfer the clear lysate into a new tube. Measure protein concentration if desires.

3. Add 5-50 µl of the clear lysate into 96 wells depending on Cathepsin H activity in the sample. Duplicate if desired. Add CH Cell Lysis Buffer to total 50 µl each well. Do a negative control as background using 50 µl CH Cell Lysis Buffer only without lysate.

Note (Optional):
For **negative control**, add 2 µl of CH Inhibitor into samples

4. Prepare Reaction Master Mix. For each reaction:

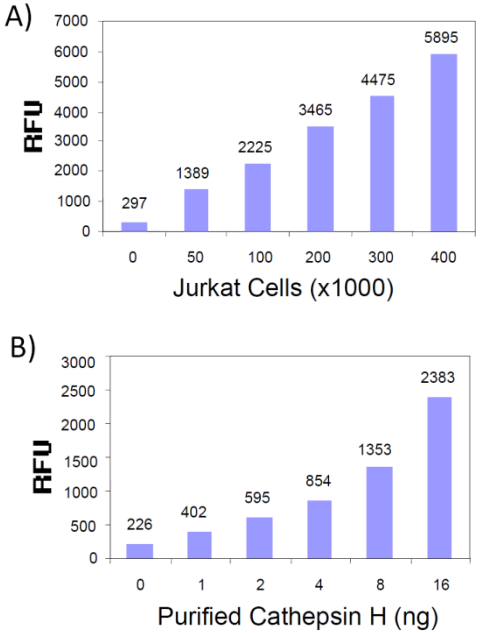
CH Reaction Buffer	50 µl
CH Substrate R-AFC	2 µl

Mix well. Add 52 µl of the master mix into each reaction. Mix and incubate at 37°C for 1-2 hours or longer. The signal increases as incubation time increases.

5. Read samples with a Fluorometer equipped with a 400-nm excitation and 505-nm emission filters.

5. Data Analysis

Cathepsin H activity can be expressed by Relative Fluorescence Units (RFU)/mg protein/min or RFU/million cells/min. If desired, cathepsin H activity can be determined by generating a standard curve using free AFC under your assay conditions.



Cathepsin H assays were performed using various numbers of Jurkat cells (A) or various amounts of purified human liver cathepsin H (B), as indicated. Results were analyzed using a fluorescence plate reader (Ex/Em = 400/505 nm) as described in 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)
	Old DTT used	Always use freshly thawed DTT in the cell lysis buffer
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

Problem	Reason	Solution
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
	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

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
www.abcam.com

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

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

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