PRODUCT INFORMATION & MANUAL

Cathepsin K Assay Kit (Fluorometric)

NBP2-54842

For research use only.

Not for diagnostic or therapeutic procedures.

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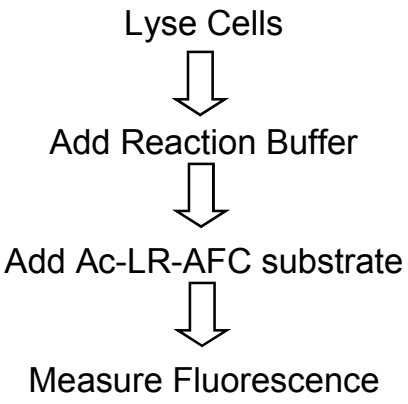
1. Overview

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

Cathepsin K Assay Kit (Fluorometric) is a fluorescence-based assay that utilizes the preferred cathepsin-K substrate sequence LR labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin-K will cleave the synthetic substrate LR-AFC to release free AFC. The released AFC can easily be quantified using a fluorometer or fluorescence plate reader.

The Cathepsin K 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
Cathepsin K Cell Lysis Buffer	25 mL
Cathepsin K Reaction Buffer	5 mL
Cathepsin K Substrate Ac-LR-AFC (10 mM)	0.2 mL
Cathepsin K Inhibitor (1 mM)	20 µL

Store kit at -20°C Protect Cathepsin K Substrate Ac-LR-AFC from light. Store Cathepsin K Cell Lysis Buffer and Cathepsin K Reaction Buffer at +4°C after opening. 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 (1-5 x 10^6) by centrifugation.

   Note:
   Use 50-200 µg cell lysates (in 50 µl of Cathepsin K Cell Lysis Buffer) if protein concentration has been measured.

2. Lyse cells in 50 µl of chilled Cathepsin K Cell Lysis Buffer. Incubate cells on ice for 10 min.

3. Centrifuge at top speed in a microcentrifuge for 5 min, transfer the supernatant to a new tube. Add 50 µl of cell lysate to a 96-well plate.

4. Add 50 µl of Cathepsin K Reaction Buffer to each sample.

5. Add 2 µl of the 10 mM Ac-LR-AFC substrate (200 µM final concentration).

   Note:
   For negative control, add 2 µl of Cathepsin K Inhibitor (Optional).

6. Incubate at 37°C for 1-2 hour.

7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. You may also perform the entire assay directly in a 96-well plate.

Fold-increase in Cathepsin K activity can be determined by comparing the relative fluorescence units (RFU) with the level of the uninduced control or the negative control sample.

If desired, the units of Cathepsin K can be determined by generating a standard curve using free AFC under your assay conditions.

5. 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