Product Information

Cathepsin L Activity Fluorometric Assay Kit

I. Kit Contents:

Component	K2153-100 100 assays	Cap Color	Part Number
CL Buffer	30 ml	WM	K2153-C-1
DTT	100 µl	Blue	K2153-C-2
Cathepsin L Positive Control	1 vial	Green	K2153-C-3
CL Substrate Ac-FR-AFC (10 mM)	0.2 ml	Brown	K2153-C-4
CL Inhibitor (1 mM)	20 µl	Red	K2153-C-5

II. Introduction:

Apoptosis is often mediated by the traditional caspase-mediated cleavage cascade. In addition, alternative proteolytic enzymes such as the lysosomal cathepsin proteases can also initiate or propagate proapoptotic signals. Cathepsins are lysosomal proteases that play an important role in mammalian cellular turnover such as bone resorption. Cathepsins are often used as sensitive markers in a variety of toxicological investigations. Cathepsin L is a lysosomal cysteine protease belonging to the peptidase C1 family and is involved in the initiation of protein degradation.

The Cathepsin L Activity Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of cathepsin L activity based on fluorometric method. The assay utilizes the preferred cathepsin-L substrate sequence FR labeled with AFC (amino-4-trifluoromethyl coumarin). While cleavage of the synthetic substrate FR-AFC by cathepsin-L in cell lysates or other samples, free AFC emits a yellow-green fluorescence (λmax = 505 nm) that can be easily quantified using a fluorecence microtiter plate reader or a fluorometer.

III. Cathepsin L Assay Protocol:

1. Collect cells (1 - 5 x 10^6) by centrifugation.

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

2. Lyse cells in 50 µl of chilled CL 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. If a positive control well is desired, add 45 µl CL Buffer and 5 µl of reconstituted positive control to a separate well.

5. Add 50 µl of CL Reaction Buffer to each sample.

6. Add 1 µl of DTT to each well.

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

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

8. Incubate at 37°C for 1 - 2 hour.

9. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may also perform the entire assay directly in a 96-well plate.Fold-increase in Cathepsin L 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 L can be determined by generating a standard curve using free AFC under your assay conditions.

IV. Storage and Stability:

Store kit at -20°C (Store CL Buffer at 4°C after opening). Protect CK Substrate Ac-FR-AFC from light.
All reagents are stable for 6 months under proper storage conditions. Dissolve positive control in 25 µl CL Buffer.

General Troubleshooting Guide for Cathepsin Fluorometric Kits:

Problems	Cause	Solution
Assay not working	• Cells did not lyse completely • Experiment was not performed at optimal time after apoptosis induction • Plate read at incorrect wavelength	• Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet • Perform a time-course induction experiment for apoptosis • Check the wavelength listed in the datasheet and the filter settings of the instrument
High Background	• Increased amount of cell lysate used • Increased amounts of components added due to incorrect pipetting • Incubation of cell samples for extended periods • Use of expired kit or improperly stored reagents • Contaminated cells	• Refer to datasheet and use the suggested cell number to prepare lysates • Use calibrated pipettes • Refer to datasheet and incubate for exact times • Always check the expiry date and store the individual components appropriately • Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	• Cells did not initiate apoptosis • Very few cells used for analysis • Use of samples stored for a long time • Incorrect setting of the equipment used to read samples • Allowing the reagents to sit for extended times on ice	• Determine the time-point for initiation of apoptosis after induction (time-course experiment) • Refer to datasheet for appropriate cell number • Use fresh samples or aliquot and store and use within one month for the assay • Refer to datasheet and use the recommended filter setting • Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	• Uneven number of cells seeded in the wells • Samples prepared in a different buffer • Adherent cells dislodged and lost at the time of experiment • Cell/ tissue samples were not completely homogenized • Samples used after multiple freeze-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Seed only equal number of healthy cells (correct passage number) • Use the cell lysis buffer provided in the kit • Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters • Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope • Aliquot and freeze samples, if needed to use multiple times • Troubleshoot as needed • Use fresh samples or store at correct temperatures until use
Unanticipated results	• Measured at incorrect wavelength • Cell samples contain interfering substances	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit (run proper controls)
General issues	• Improperly thawed components • Incorrect incubation times or temperatures • Incorrect volumes used • Air bubbles formed in the well/tube • Substituting reagents from older kits/ lots	• Thaw all components completely and mix gently before use • Refer to datasheet & verify the correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly • Pipette gently against the wall of the well/tubes

	• Use of a different 96-well plate	• Use fresh components from the same kit • Fluorescence: Black plates; Absorbance: Clear plates
Note# The most probable cause is listed under each section. Causes may overlap with other sections.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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