

Cathepsin B Activity Fluorometric Assay Kit

(Catalog #BN00355; 100 assays; Store kit at -20° C)

I. Introduction:

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. The Cathepsin-B Activity Assay kit is a fluorescence-based assay that utilizes the preferred cathepsin-B substrate sequence RR labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin-B will cleave the synthetic substrate RR-AFC to release free AFC. The released AFC can easily be quantified using a fluorometer or fluorescence plate reader. The cathepsin-B assay is simple, straightforward, and can be adapted to 96-well plate assays. Assay conditions have been optimized to obtain the maximal activity.

Fold-increase in Cathepsin B 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 B can be determined by generating a standard curve using free AFC under your assay conditions. Free AFC is available from Assay Genie.

II. Kit Contents:

Components	BN00355	Cap Code	Part No.
CB Cell Lysis Buffer	25 ml	WM	BN00355-1
CB Reaction Buffer	5 ml	NM	BN00355-2
CB Substrate Ac-RR-AFC (10 mM)	0.2 ml	Brown	BN00355-3
CB Inhibitor (1 mM)	20 μ l	Red	BN00355-4

III. Storage and Stability:

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

IV. Cathepsin B Assay Protocol:

- Collect cells ($1-5 \times 10^6$) by centrifugation.
Note: Use 50-200 μ g cell lysates (in 50 μ l of Cell lysis Buffer) if protein concentration has been measured.
- Lyse cells in 50 μ l of chilled CB Cell Lysis Buffer. Incubate cells on ice for 10 min.
- Centrifuge at top speed in a microcentrifuge for 5 min, transfer the supernatant to a new tube. Add 50 μ l of cell lysate to a 96-well plate.
Note: We recommend using a flat bottom, opaque, white or black 96-well plate for enhanced sensitivity.
- Add 50 μ l of CB Reaction Buffer to each sample.
- Add 2 μ l of the 10 mM CB Substrate Ac-RR-AFC (200 μ M final concentration).
Note: For negative control, add 2 μ l of CB Inhibitor (Optional).
- Incubate at 37° C for 1-2 hour.
- 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.

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE FOR CATHEPSIN FLUOROMETRIC KITS:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Cells did not lyse completely • Experiment was not performed at optimal time after apoptosis induction • Plate read at incorrect wavelength 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Cell samples contain interfering substances 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit (run proper controls)
General issues	<ul style="list-style-type: none"> • Improperly thawed components • Incorrect incubation times or temperatures • Incorrect volumes used • Air bubbles formed in the well/tube • Substituting reagents from older kits/ lots • Use of a different 96-well plate 	<ul style="list-style-type: none"> • 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 fresh components from the same kit • Fluorescence: Black plates; Absorbance: Clear plates
<p>Note: The most probable cause is listed under each section. Causes may overlap with other sections.</p>		