MA-0168

# Cathepsin B Activity Assay

**(100 assays; Fluorometric, Ex/Em = 380/480 nm, Store kit at -20°C)**

## Introduction:

Cathepsin B, a lysosomal cysteine protease, plays a crucial role in apoptosis, the programmed cell death essential for maintaining cellular homeostasis and eliminating damaged or unnecessary cells. Located within lysosomes, cathepsin B participates in the intricate molecular cascade that orchestrates apoptosis. During apoptosis, various signals activate the intrinsic (mitochondrial) pathway, triggering the release of pro-apoptotic factors. Cathepsin B contributes to this process by cleaving Bid, a pro-apoptotic Bcl-2 family member, into its truncated form, tBid. TBid then translocates to the mitochondria, leading to the release of cytochrome c and subsequent activation of caspases, key executioners of apoptosis. Additionally, cathepsin B has been implicated in lysosomal membrane permeabilization (LMP), a phenomenon associated with apoptosis. LMP facilitates the release of cathepsin B into the cytoplasm, amplifying its pro-apoptotic effects. Once in the cytoplasm, cathepsin B can directly activate caspases or cleave anti-apoptotic proteins, promoting the irreversible commitment to cell death. The dysregulation of cathepsin B has been linked to various pathological conditions, including neurodegenerative diseases and cancer. Consequently, understanding the intricate interplay of cathepsin B in apoptosis provides valuable insight into potential therapeutic strategies targeting these conditions. AkrivisBio's Cathepsin B Assay is a simple, sensitive fluorescence-based method which depends upon cleavage of the specific Cathepsin B substrate, RR-AFC.

## Assay Components:

| | | | |
|---|---|---|---|
| Cell Lysis Buffer | 25 ml | WM | MA-0168A |
| Reaction Buffer | 5 ml | NM | MA-0168B |
| Ac-RR-AFC | 0.2 ml | Amber | MA-0168C |
| Inhibitor | 20 µl | Red | MA-0168D |
| Cathepsin B | Lyoph | Green | MA-0168E |
| AFC Standard | 100 µl | Yellow | MA-0168F |

## Storage and Handling:

Store unopened assay kit at -20°C. Bring all components to room temperature before use.
**Cell Lysis Buffer, Reaction Buffer**: Ready to use as supplied. Store at 4°C.
**Ac-RR-AFC, AFC Standard:** Ready to use as supplied. Ac-RR-AFC comes as a 10 mM solution in DMSO. AFC Standard is 1 mM in DMSO. Protect from light and store at -20°C.
**Cathepsin B:** Reconstitute with 25 µl DI $H_2O$. Aliquot to convenient portions and store at -80°C to avoid repeated freeze/thaw cycles.
**Inhibitor:** Ready to use as supplied. Comes as a 1 mM solution in DMSO. Store at -20°C.

## Assay Protocol:

**1. Standard Curve (OPTIONAL):**
Dilute AFC Standard to 10 µM by adding 10 µl to 990 µl of Reaction Buffer. Add 0, 20, 40, 60, 80, 100 µl to a series of wells in a 96-well plate. Adjust all wells to 100 µl with Reaction Buffer, giving 0 – 20 – 40 – 60 – 80 – 100 pmoles AFC in 100 µl, respectively (concentrations are 0 – 20 – 40 – 60 – 80 – 100 µM).

**2. Sample Preparation:**
- **A.** Culture the cells of choice. Induce apoptosis. Collect cells (1 - 2 x $10^6$) by gentle centrifugation t 100-200 X g for 5-10 minutes.
- **B.** Lyse cells in 50 µl of chilled Cell Lysis Buffer on ice for 10 minutes.

**Note:** If the protein concentration has already been measured, use cell lysate at 1-2 mg/ml in Cell Lysis Buffer.

- **C.** Centrifuge at ~ 20,000 X g for 5 minutes, then transfer the clear supernatant to a fresh tube. Add up to 50 µl of cell lysate per well in a 96-well plate.
- **D.** Add 50 µl of Reaction Buffer to each sample and preincubate at 37° for 5-15 minutes.
- **E.** Add 2 µl of the 10 mM CB Substrate Ac-RR-AFC.
- **F.** If a negative control is needed, add 2 µl of Inhibitor.

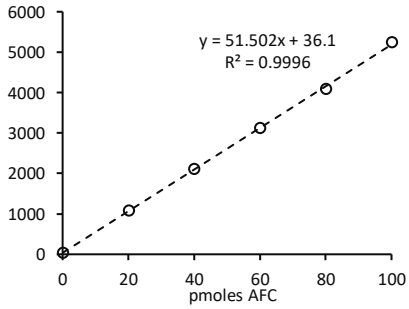
**3. Positive Control (Optional):** Add 2 µl of reconstituted Positive Control to wells and adjust volume to 50 µl with Reaction Buffer.

**4. Measurement:**
Monitor the fluorescence in a plate reader at 380/480 nm at 37°C for 1-2 hours.

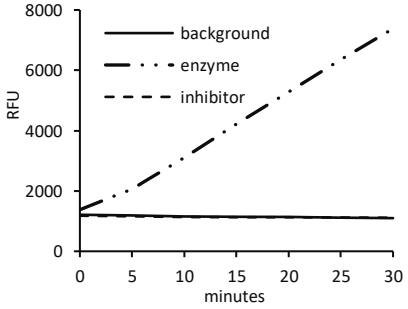
**5. Typical Results:**

| pmole Standard | Standard Raw Values RFU | Background Corrected Values RFU |
|---|---|---|
| 0 | 32.0 | 0 |
| 20 | 1086.2 | 1054.2 |
| 40 | 2104.4 | 2072.4 |
| 60 | 3118.1 | 3086.1 |
| 80 | 4088.1 | 4054.1 |
| 100 | 5238.4 | 5206.4 |

**Standard Curve**




**Positive control ± Inhibitor control**




**6.** The increase in Cathepsin B activity is determined by comparing the relative fluorescence units (RFU) with the level of the uninduced control or the negative control sample. If desired, precise Cathepsin B activity can be determined with respect to a standard curve. Subtract the zero standard from all other standards. Plot the Standard Curve and determine its slope (RFU/pmole). Identify a linear portion of the Sample enzyme activity and determine the slope of the liner portion (RFU/minute). Divide the Cathepsin B enzyme activity slope by the slope of the Standard Curve to convert to pmoles/minute in the well.

To convert back to enzyme activity in the original sample:
- A. Divide the activity determined in the well by the volume in µl of sample added to the well = pmole/min (µU) per µl of sample.
- B. Multiply the pmole/min per µl of sample.in A. by the total volume of extract obtained in Step 2C above = total activity (µU) of sample.
- C. Divide total activity in µU of sample by mg tissue or # of cells used to prepare sample = µU Cathepsin B enzyme activity/mg of sample (or per # of cells used, etc.)

***For Research Use Only. Not to be used for diagnostic or therapeutic purposes***

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