

# Cathepsin B Activity Assay Kit

(Catalog #K140-100; 100 assays; Store kit at  $-20^{\circ}\text{C}$ )

## 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.

## II. Kit Contents:

Components	K140-100	Color Code
	100 assays	Cap Color
CB Cell Lysis Buffer	25 ml	WM
CB Reaction Buffer	5 ml	NM
CB Substrate Ac-RR-AFC (10 mM)	0.2 ml	Brown
CB Inhibitor (1 mM)	20 $\mu\text{l}$	Red

## III. Storage and Stability:

- Store kit at  $-20^{\circ}\text{C}$  (Store CB Cell Lysis Buffer and CB Reaction Buffer at  $4^{\circ}\text{C}$  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  $\mu\text{g}$  cell lysates (in 50  $\mu\text{l}$  of Cell lysis Buffer) if protein concentration has been measured.

- Lyse cells in 50  $\mu\text{l}$  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  $\mu\text{l}$  of cell lysate to a 96-well plate.
- Add 50  $\mu\text{l}$  of CB Reaction Buffer to each sample.
- Add 2  $\mu\text{l}$  of the 10 mM CB Substrate Ac-RR-AFC (200  $\mu\text{M}$  final concentration).

Note: For negative control, add 2  $\mu\text{l}$  of CB Inhibitor (Optional).

- Incubate at  $37^{\circ}\text{C}$  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.

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 BioVision (Cat.# 1077-100).

## V. Related Products:

### Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Additional Apoptosis Kits & Reagents

### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

### Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione Fluorometric & Colorimetric Assay Kits
- Nitric Oxide Fluorometric & Colorimetric Assay Kits

### Signal Transduction

- Camp & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

### Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

### Molecular Biology & Reporter Assays

- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit

### Antibodies & Recombinant Proteins (many)