

Cathepsin L Activity Assay Kit

(Catalog #K142-100; 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-L Activity Assay kit is a fluorescence-based assay that utilizes the preferred cathepsin-L substrate sequence FR labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin-L will cleave the synthetic substrate FR-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.

II. Kit Contents:

Components	K142-100	Color Code	Part Number
	100 assays	Cap Color	Component
CL Cell Lysis Buffer	25 ml	WM	K142-100-1
CL Reaction Buffer	5 ml	NM	K142-100-2
CL Substrate Ac-FR-AFC (10 mM)	0.2 ml	Brown	K142-100-3
CL Inhibitor (1 mM)	20 µl	Red	K142-100-4

III. Storage and Stability:

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

IV. Cathepsin L Assay Protocol:

- Collect cells ($1-5 \times 10^6$) by centrifugation.
Note: Use 50-200 µg cell lysates (in 50 µl of CL Cell Lysis Buffer) if protein concentration has been measured.
- Lyse cells in 50 µl of chilled CL 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 µl of cell lysate to a 96-well plate.
- Add 50 µl of CL Reaction Buffer to each sample.
- 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).
- 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.

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. 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)