

Cathepsin D Activity Assay Kit

(Catalog #K143-100; 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-D Activity Assay kit is a fluorescence-based assay that utilizes the preferred cathepsin-D substrate sequence GKPIFFRLK(Dnp)-D-R-NH₂ labeled with MCA. Cell lysates or other samples that contain cathepsin-D will cleave the synthetic substrate to release fluorescence, which can then easily be quantified using a fluorometer or fluorescence plate reader at Ex/Em = 328/460 nm. The cathepsin-D 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	100 Assays	Cap Color	Part Number
CD Cell Lysis Buffer	25 ml	WM	K143-100-1
CD Reaction Buffer	5 ml	NM	K143-100-2
CD Substrate (1mM)	0.2 ml	Brown	K143-100-3

III. Storage and Stability:

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

IV. Cathepsin D Assay Protocol:

- Collect cells (1×10^6) by centrifugation.
- Lyse cells in 200 μl of chilled CD Cell Lysis Buffer. Incubate cells on ice for 10 min.
- Centrifuge for 5 min at top speed. Transfer the clear cell lysate into a labeled new tube.
- Add 5-50 μl of the cell lysate (or ~ 1 -10 ng of purified Cathepsin D protein samples) into each well in a 96-well plate. Bring the total volume to 50 μl with CD Cell Lysis Buffer.
- Prepare a master assay mix, for each assay:
 - 50 μl of Reaction Buffer
 - 2 μl of Substrate
- Mix the master assay mix. Add 52 μl of the master assay mix into each assay wells. Mix well. Incubate at 37°C for 1-2 hour.
- Read samples in a fluorometer equipped with a 328-nm excitation filter and 460-nm emission filter.

Cathepsin D activity can be expressed by the relative fluorescence units (RFU) per million cells, or RFU per microgram protein of your sample, or RFU fold increase of treated samples vs the untreated control or the negative control sample.

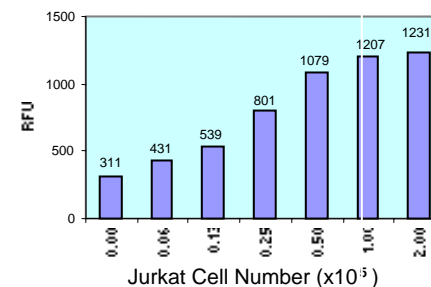


Figure 1. Cathepsin D assays were performed using various numbers of Jurkat Cells as indicated. Results were analyzed by fluorescence plate reader according to the kit instructions.

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- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA Vectors

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
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- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
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- DNA Damage Quantification Kit
- Glutathione Fluorometric & Colorimetric Assay Kits
- Nitric Oxide Fluorometric & Colorimetric Assay Kits
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- SOD Assay Kit and more

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