

Cathepsin H Activity Assay Kit

(Catalog #K145-100; 100 assays; Store 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-H Activity Assay kit is a fluorescence-based assay that utilizes the preferred cathepsin-H substrate Arginine labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin-H will cleave the synthetic substrate R-AFC to release free AFC. The released AFC can easily be quantified using a fluorometer or fluorescence plate reader. The cathepsin-H 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 Code	Part No.
CH Cell Lysis Buffer	25 ml	WM	K145-100-1
CH Reaction Buffer	5 ml	NM	K145-100-2
CH Substrate R-AFC (10 mM)	0.2 ml	Amber	K145-100-3
CH Inhibitor (1 mM)	20 µl	Red	K145-100-4

III. Storage and Stability:

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

IV. Cathepsin H Assay Protocol:

- Collect cells (10⁶) by centrifugation. If the sample is tissue, use 10 mg tissue. Lyse cells or tissue in 50 µl of chilled CH Cell Lysis Buffer. Incubate cells on ice for 10 minutes. Vortex for 5 minutes.
- Centrifuge 13000 rpm for 5 min in bench-top micro-centrifuge to remove insoluble materials. Transfer the clear lysate into a new tube. Measure protein concentration if desired.
- Add 5-50 µl of the clear lysate into 96 wells depend on cathepsin H activity in the sample. Duplicate if desired. Add CH Cell Lysis Buffer to total 50 µl each well. Do a negative control as background using 50 µl CH Cell Lysis Buffer only without lysate.
Note: For negative control, add 2 µl of CH Inhibitor into samples (Optional).
- Prepare Reaction master mix. For each reaction:
50 µl of CH Reaction Buffer
2 µl of CH Substrate R-AFC
Mix well.
- Add 52 µl of the master mix into each reaction.

- Mix and Incubate at 37°C for 1-2 hour or longer. Signal increase as incubation time increase.
- Read samples with a Fluorometer equipped with a 400-nm excitation and 505-nm emission filters.

Cathepsin H activity can be expressed by Relative Fluorescence Units (RFU)/mg protein/min or RFU/million cells/min. If desired, cathepsin H activity can be determined by generating a standard curve using free AFC under your assay conditions. Free AFC is available from BioVision (Cat. # 1077-100).

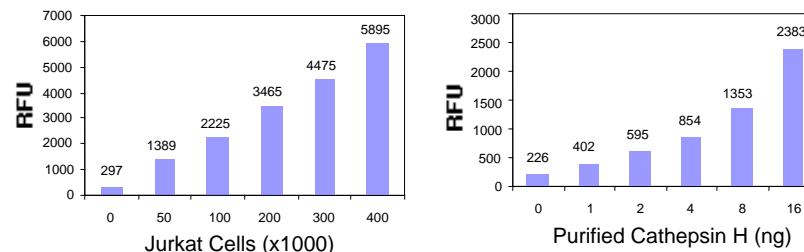


Figure 1. Cathepsin H Activity Assay. Cathepsin H assays were performed using various numbers of Jurkat cells (A) or various amounts of purified human liver cathepsin H (B), as indicated. Results were analyzed using a fluorescence plate reader (Ex/Em = 400/505 nm) as described in the kit instructions.

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
- Live-Dead staining Kit
- ADP/ATP Ratio Assay Kit
- ATP Cell Viability Assay Kit