Cathepsin L Activity Assay Kit

Catalog Number KA0770

100 assays

Version: 02

Intended for research use only

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Introduction

Background

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.

General Information

Materials Supplied

List of component

Component	Amount
CL Cell Lysis Buffer	25 ml
CL Reaction Buffer	5 ml
CL Substrate Ac-RR-AFC (10 mM)	0.2 ml
CL Inhibitor (1 mM)	20 µl

Storage Instruction

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.

Precaution of Use

For research use only! Not to be used on humans.

Assay Protocol

Assay Procedure

1. Collect cells (1-5 x 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.
2. Lyse cells in 50 µl of chilled CL Cell Lysis Buffer. Incubate cells on ice for 10 min.
3. 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.
4. Add 50 µl of CL Reaction Buffer to each sample.
5. Add 2 µl of the 10 mM CL Substrate Ac-FR-AFC (200 µM final concentration).
   Note: For negative control, add 2 µl of Cathepsin L Inhibitor (Optional).
6. Incubate at 37°C for 1-2 hour.
7. 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.