Technical Bulletin

Cathepsin D Assay Kit

Catalog Number MAK383

Product Description

Apoptosis can be mediated by mechanisms other than the traditional caspase-mediated cleavage cascade. There is growing recognition that alternative proteolytic enzymes such as 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 GKPILFFRLK(Dnp)-DR-NH_2) labeled with 7-Methoxycoumarin-4-acetic acid (MCA). Cell lysates or other samples that contain cathepsin D cleave the synthetic substrate to release fluorescence, which can be quantified fluorometrically at λ_{Ex} = 328 nm/ λ_{Em} = 460 nm. The Cathepsin D assay is simple and straightforward. Assay conditions have been optimized to obtain the maximal activity.

The kit is suitable for the detection of Cathepsin D activity in cell lysates.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- CD Cell Lysis Buffer 25 mL
  Catalog Number MAK383A
- CD Reaction Buffer 5 mL
  Catalog Number MAK383B
- CD Substrate (1 mM) 200 µL
  Catalog Number MAK383C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- Flat bottom, opaque, white or black 96-well plate for enhanced sensitivity. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

CD Cell Lysis Buffer and CD Reaction Buffer: Store at 2-8 °C, protected from light after opening.

CD Substrate (1 mM): Protect from light.

Procedure

Sample Preparation

1. Induce sample cells to increase Cathepsin D activity by adding test chemical. For unknown test chemicals, it is recommended to prepare several cell cultures and add different concentrations of test chemical.
2. Prepare a separate cell culture with **no** test chemical inducer for use as an Uninduced Control.
3. Collect cells (1×10^6 cells) by centrifugation.
4. Lyse cells in 200 µL of chilled CD Cell Lysis Buffer.
5. Incubate cells on ice for 10 minutes.
6. Centrifuge at top speed in a microcentrifuge for 5 minutes.
7. Transfer the supernatants to new tubes.
8. Add 5-50 µL of each cell lysate, including Uninduced Control cell lysate to a 96-well plate. Bring the total volume to 50 µL with CD Cell Lysis Buffer. Note: If not running multiple sample cell lysates with different amounts of test chemical, use ~1-10 ng of purified Cathepsin D protein samples (diluted to a total volume of 50 µL) if protein concentration has been measured.

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 52 µL of Reaction Mix according to Table 1. Mix well.

Table 1.
Preparation of Reaction Mix

Reagent	Volume
CD Reaction Buffer	50 µL
CD Substrate (1mM)	2 µL

2. Add 52 µL of Reaction Mix into each assay well. Mix well.
3. Incubate plate at 37 °C for 1-2 hours.

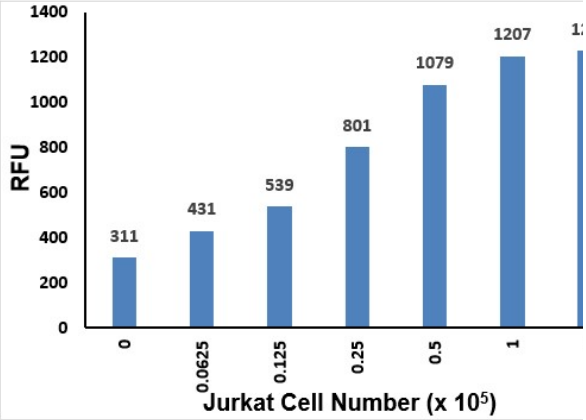
Measurement

After incubation, measure fluorescence (RFU) for all wells at λ_{Ex} = 328 nm/λ_{Em} = 460 nm.

Results

Relative Cathepsin D activity can be expressed as relative fluorescence units (RFU) per million cells, as RFU per microgram protein of sample, or as a multiple-fold increase in RFU of Induced Samples versus the RFU of an Uninduced Control or a 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 protocol.



Frequently Asked Questions

What is the preferential cleavage site for the substrate in the Cathepsin D activity kit?

For the substrate GKPILFFRLK(Dnp)-DR-NH_2) labeled with MCA, cleavage occurs at the Phe-Phe amide bond resulting in enhanced fluorescence.

Does the kit work with bacteria or yeast cells?

The kit has been standardized for mammalian cells only.

Can an alternate buffer be used for sample preparation (cell lysis, sample dilutions, etc.)?

The Cathepsin D Activity Assay buffers are optimized for the reaction they are designed for. The buffers not only contain detergents for efficient lysis of cells/tissue, but also contain proprietary components required for the further reactions. Therefore, it is highly recommended to use the buffers provided in the kit for the best results.

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