Technical Bulletin

Cathepsin B Activity Assay Kit

Catalog Number MAK387

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 B Activity Assay kit uses a fluorescence-based assay that utilizes the preferred cathepsin B substrate sequence RR labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin B cleave the synthetic substrate RR-AFC to release free AFC. The released AFC can easily be quantified fluorometrically. The cathepsin B assay is simple and straightforward. Assay conditions have been optimized to obtain the maximal activity.

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

Components

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

- CB Cell Lysis Buffer 25 mL
  Catalog Number MAK387A
- CB Reaction Buffer 5 mL
  Catalog Number MAK387B
- CB Substrate Ac-RR-AFC (10 mM) 200 µL
  Catalog Number MAK387C
- CB Inhibitor (1 mM) 20 µL
  Catalog Number MAK387D

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
- 7-Amino-4-(trifluoromethyl)coumarin (AMC) (Catalog Number 248924) (optional)

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.

CB Cell Lysis Buffer and CB Reaction Buffer: Store at 2-8 °C after opening.

CB Substrate Ac-RR-AFC (10 mM): Protect from light.

Procedure

Sample Preparation

1. Induce sample cells to increase Cathepsin B 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 (1-5 × 10^6 cells) by centrifugation.
4. Lyse cells in 50 µL of chilled CB 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 50 µL of each cell lysate, including Uninduced Control cell lysate, to a 96-well plate. Note: If not running multiple sample cell lysates with different amounts of test chemical, use 50-200 µg of cell lysates (diluted to a total volume of 50 µL of CB Cell Lysis Buffer) if protein concentration has been measured.

Negative Cell Lysate Control (Optional)

Add 50 µL of Uninduced Control cell lysate and 2 µL of CB Inhibitor (1 mM) to designated well.

Assay Procedure

1. Add 50 µL of CB Reaction Buffer to all Induced Sample and Control (Uninduced and optional Negative Cell Lysate) wells.
2. Add 2 µL of the 10 mM CB Substrate Ac-RR-AFC (200 µM final concentration) to each well.
3. Incubate at 37 °C for 1-2 hours.

Measurement

Measure the fluorescence (RFU) at λ_{Ex} = 400 nm/λ_{Em} = 505 nm.

Results

Relative Cathepsin B activity can be determined by comparing the Induced Sample relative fluorescence units (RFU) with the RFU values of the Uninduced Control or the Negative Control. If desired, the units of cathepsin B can be determined by generating a standard curve using free AFC (not included) under the assay conditions.

Frequently Asked Questions:

Can the kit work on 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 B Activity Assay Kit assay buffers are optimized for the reactions the kit is 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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