Technical Bulletin

# Cathepsin L Activity Assay Kit

**Catalog Number MAK401**

## 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 L Activity Assay kit uses 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 cleave the synthetic substrate FR-AFC to release free AFC. The released AFC can easily be quantified fluorometrically. The cathepsin L assay is simple and straightforward. Assay conditions have been optimized to obtain the maximal activity.

The kit is suitable for the measurement of relative cathepsin L activity in cell lysates.

## Components

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

- CL Buffer 30 mL
  Catalog Number MAK401A
- DTT 100 µL
  Catalog Number MAK401B
- Cathepsin L Positive Control 1 vial
  Catalog Number MAK401C
- CL Substrate Ac-FR-AFC (10 mM) 200 µL
  Catalog Number MAK401D
- CL Inhibitor (1 mM) 20 µL
  Catalog Number MAK401E

## Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White, opaque or black flat-bottom 96-well plates. 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, protected from light.

## Preparation Instructions

Briefly centrifuge small vials prior to opening.

CL Buffer: Ready to use. Store at 2-8 °C after opening. Chill an appropriate amount of CL Buffer for use in Sample Preparation.

Cathepsin L Positive Control: Reconstitute the vial in 25 µL of CL Buffer.

## Procedure

Sample Preparation

1. Induce sample cells to increase Cathepsin L 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 each set of cells (1-5 × $10^6$ cells) by centrifugation.
4. Lyse each set of cells in 50 µL of chilled CL 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 CL Buffer) if protein concentration has been measured.

Positive CL Control (Optional)

Add 45 µL of CL Buffer and 5 µL of reconstituted Cathepsin L Positive Control to designated well.

Negative Cell Lysate Control (Optional)

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

Assay Procedure

1. Add 50 µL of CL Buffer to all Induced Sample and Control (Uninduced, optional Positive CL, and optional Negative Cell Lysate) wells.
2. Add 1 µL of DTT to each well.
3. Add 2 µL of CL Substrate Ac-FR-AFC (10 mM) to each well EXCEPT the Negative Control well.
4. Incubate at 37 °C, protected from light for 1-2 hours.

Measurement

After incubation, measure the fluorescence (RFU) at $\lambda_{Ex}$ = 400 nm/$\lambda_{Em}$ = 505 nm.

## Results

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

**Figure 1.**
Measurement of recombinant human Cathepsin L activity using an AFC standard curve. Cathepsin L activity is reported as nmol of AFC released per minute.

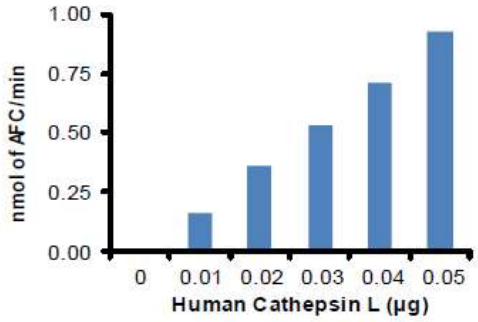



**Figure 2.**
Measurement of Cathepsin L activity in HepG2 lysate using an AFC standard curve. Cathepsin L activity is reported as nmol of AFC released per minute.

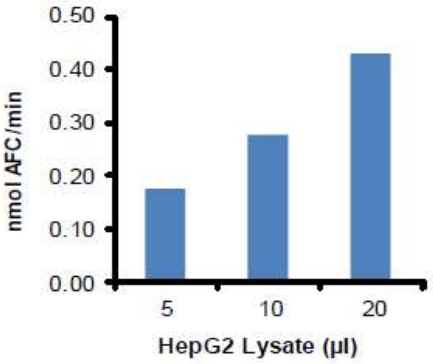
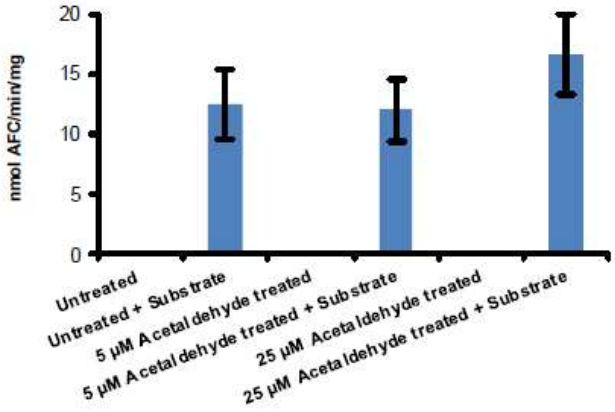



**Figure 3.**
Measurement of Cathepsin L activity in untreated (uninduced cells) and treated (cells induced with 5 or 25 µM Acetaldehyde) HepG2 lysates. Lysate without the addition of substrate was used as background control. Free AFC was used to obtain AFC standard curve. The protein amount in lysate obtained after treatment with 25 µM of acetaldehyde was lower as compared to untreated or 5 µM treated cells. Assays were performed according to the kit protocol.



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