

## Technical Bulletin

## Cathepsin S Assay Kit

## Catalog Number MAK403

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

The kit is suitable for the measurement of cathepsin S in cell lysates.

## Components

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

- CS Cell Lysis Buffer 25 mL  
Catalog Number MAK403A
- CS Reaction Buffer 5 mL  
Catalog Number MAK403B
- CS Substrate Z-VVR-AFC (10 mM) 200  $\mu$ L  
Catalog Number MAK403C
- CS Inhibitor (1 mM) 20  $\mu$ L  
Catalog Number MAK403D

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 (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.

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

CS Substrate Z-VVR-AFC (10 mM): Protect from light.

## Procedure

All samples and standards should be run in duplicate.

### Sample Preparation

1. Induce sample cells to increase Cathepsin S 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 ( $1-5 \times 10^6$  cells ) by centrifugation.
4. Lyse cells in 50  $\mu$ L of chilled CS 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  $\mu$ L 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  $\mu$ g of cell lysates (diluted to a total volume of 50  $\mu$ L of CS Cell Lysis Buffer) if protein concentration has been measured.

### Negative Cell Lysate Control (Optional)

Add 50  $\mu$ L of Uninduced Control cell lysate and 2  $\mu$ L of CS Inhibitor (1 mM) to designated well.

### Assay Procedure

1. Add 50  $\mu$ L of CS Reaction Buffer to all Induced Sample and Control (Uninduced and optional Negative Cell Lysate) wells.
2. Add 2  $\mu$ L of CS Substrate Z-VVR-AFC (10 mM) to each well.
3. 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 S 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 S can be determined by generating a standard curve using free AFC (not included) under the assay conditions.



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