



## Cathepsin D Assay Kit

**Cat. #: CB005**  
**(100 tests)**

**GenBank access number:** CAA28955.1

### Kit components:

Cell Lysis Buffer: 25 ml

Reaction Buffer: 5 ml

Substrate (1 mM): 0.2 ml, GKPILFFRLK(Dnp)-D-R-NH<sub>2</sub>-MCA

### 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 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-D Activity Assay kit is a fluorescence-based assay that utilizes the preferred cathepsin-D substrate sequence GKPILFFRLK(Dnp)-D-R-NH<sub>2</sub>) labeled with MCA. Cell lysates or other samples that contain cathepsin-D will cleave the synthetic substrate to release fluorescence, which can then easily be quantified using a fluorometer or fluorescence plate reader at Ex/Em = 328/460 nm. The cathepsin-D assay is simple, straightforward, and can be adapted to 96-well plate assays. Assay conditions have been optimized to obtain the maximal activity.

### Procedure

1. Collect cells ( $1 \times 10^6$ ) by centrifugation.
2. Lyse cells in 200  $\mu$ l of chilled Cell Lysis Buffer. Incubate cells on ice for 10 min.
3. Centrifuge for 5 min at top speed. Transfer the clear cell lysate into a labeled new tube.
4. Add 5-50  $\mu$ l of the cell lysate (or ~1-10 ng of purified Cathepsin D protein samples) into each well in a 96-well plate. Bring the total volume to 50  $\mu$ l with Cell Lysis Buffer.
5. Prepare a master assay mix, for each assay:
  - Reaction Buffer: 50  $\mu$ l
  - Substrate: 2  $\mu$ l
6. Mix the master assay mix. Add 52  $\mu$ l of the master assay mix into each assay wells. Mix well. Incubate at 37°C for 1-2 hour.
7. Read samples in a fluorometer equipped with a 328-nm excitation filter and 460-nm emission filter.

Cathepsin D activity can be expressed by the relative fluorescence units (RFU) per million cells, or RFU per microgram protein of your sample, or RFU fold increase of treated samples vs the untreated control or the negative control sample.

**Storage:** Store at  $\leq -20$  °C for up to 6 months after initial use. Avoid repeated freeze-thaw cycles.