CREATIVE BIOMART®
Assay Kit

Cathepsin K Activity Fluorometric Assay Kit

Product Information

Common Name

Cathepsin

Cat.No. Kit-0184

Product Overview

The cathepsin-K assay is simple, straightforward, and can be adapted to 96-well plate assays. Assay conditions have been optimized to obtain the maximal activity.

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-K Activity Assay kit is a fluorescence-based assay that utilizes the preferred cathepsin-K substrate sequence LR labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin-K will cleave the synthetic substrate LR-AFC to release free AFC. The released AFC can easily be quantified using a fluorometer or fluorescence plate reader.

Applications

Detects alternative proteolytic enzymes such as the lysosomal cathepsin proteases that can initiate or propagate proapoptotic signals.

Stability

Store kit at -20°C (Store CK Cell Lysis Buffer and CK Reaction Buffer at 4°C after opening). Protect CK Substrate from light. All reagents are stable for 6 months under
proper storage conditions.

Storage

-20°C

Shipping

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Fax:1-631-938-8127
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Gel Pack

Size

100 assays

Kit Components

CK Cell Lysis Buffer 25 ml
CK Reaction Buffer 5 ml
CK Substrate Ac-LR-AFC (10 mM) 0.2 ml
CK Inhibitor (1 mM) 20 ul

Detection method Fluorescence (Ex/Em 400/505)

Assay Protocol

1. Collect cells (1-5 x 10^6) by centrifugation.
Note: Use 50-200 µg cell lysates (in 50 µl of Cell lysis Buffer) if protein concentration has been measured.
2. Lyse cells in 50 µl of chilled CK Cell Lysis Buffer. Incubate cells on ice for 10 minutes.
3. Centrifuge at top speed in a microcentrifuge for 5 min, transfer the supernatant to a new tube.
Add 50 µl of cell lysate to a 96-well plate.
Note: We recommend using a flat bottom, opaque, white or black 96-well plate for enhanced sensitivity.
4. Add 50 µl of CK Reaction Buffer to each sample.
5. Add 2 µl of the 10 mM CK Substrate Ac-LR-AFC (200 µM final concentration).
Note: For negative control, add 2 µl of CK Inhibitor (Optional).
5. Incubate at 37°C for 1-2 hour.
6. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may also perform the entire assay directly in a 96-well plate.
Fold-increase in Cathepsin K activity can be determined by comparing the relative fluorescence units (RFU) with the level of the uninduced control or the negative control sample. If desired, the units of cathepsin K can be determined by generating a standard curve using free AFC under your

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assay conditions.

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