Caspase-1 Assay Kit (Fluorometric)

Catalog Number KA0728

100 assays

Version: 02

Intended for research use only

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Introduction

Background

Activation of ICE-family proteases/caspases initiates apoptosis or other cellular processes in mammalian cells. The Caspase-1 Assay Kit (Fluorometric) provides a simple and convenient means for assaying the activity of caspases that recognize the sequence YVAD. The assay is based on detection of cleavage of substrate YVAD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). YVAD-AFC emits blue light (λmax = 400 nm); upon cleavage of the substrate by caspase-1 or related caspases, free AFC emits a yellowgreen fluorescence (λmax = 505 nm), which can be quantified using a fluorometer or a fluorecence microtiter plate reader. Comparison of the fluorescence of AFC from a treated sample with an untreated control allows determination of the fold increase in caspase-1 activity.

General Information

Materials Supplied

List of component

Component	Amount
Cell Lysis Buffer	100 ml
2X Reaction Buffer	4 x 2 ml
YVAD-AFC (1 mM)	0.5 ml
DTT (1 M)	0.4 ml

Storage Instruction

Store kit at -20℃ (Store Cell Lysis Buffer and 2X Reaction Buffer at 4℃ after opening). All reagents are stable for 6 months under proper storage conditions.

Precautions for Use

- Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT to the 2X Reaction Buffer immediately before use (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).
- After thawing, store the Cell Lysis Buffer and 2X Reaction Buffer at 4℃.
- Protect YVAD-AFC from light.

Assay Protocol

Assay Procedure

1. Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture without treatment.
   Note: Active Caspase-1 can be used as a positive control.
2. Pellet 1-5 x 10^6 cells or use 50-200 µg cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer. Incubate cells on ice for 10 minutes.
4. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 1 mM YVAD-AFC substrate (50 µM final concentration). Incubate at 37℃ for 1-2 hour.
5. 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 Caspase-1 activity can be determined by comparing the results of induced samples with the level of the untreated control.