Introduction and Background

A. Overview

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **FLICE/Caspase-8 Fluorometric Assay Kits** provide a simple and convenient means for assaying the activity of caspases that recognize the sequence IETD. The assay is based on detection of cleavage of substrate IETD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). IETD-AFC emits blue light (λmax = 400 nm); upon cleavage of the substrate by FLICE or related caspases, free AFC emits a yellow-green fluorescence (λmax = 505 nm), which can be quantified using a fluorometer or a fluorecence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in FLICE activity.

B. Notice for Application of Kit

✓ This kit has been configured for research use only and is not for diagnostic and clinical use.

Material and Method

A. List of component

1. Cell Lysis Buffer 100 ml
2. 2X Reaction Buffer 4 X 2 ml
3. IETD-AFC (1mM) 500 µl
4. DTT (1 M) 400 µl

B. Stability and storage

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

C. Protocol

1. General Considerations

- 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°C.
- Protect IETD-AFC from light.

2. Assay Procedure

a. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.

b. Count cells and pellet 1-5 x 10^6 cells or use 50-200 µg cell lysates if protein concentration has been measured.

c. Resuspend cells in 50 µl of chilled Cell Lysis Buffer. Incubate cells on ice for 10 minutes.

d. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 1 mM IETD-AFCsubstrate (50 µM final concentration). Incubate at 37°C for 1-2 hour.

e. 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 FLICE activity can be determined by comparing the results of induced samples with the level of the untreated control.