Introduction and Background

A. Overview

Caspases have been shown to play a crucial role in apoptosis induced by various deleterious and physiologic stimuli. Inhibition of caspases can delay apoptosis, implicating a potential role in drug screening efforts. The Caspase-1 Inhibitor Drug Screening Kit provides an effective means for screening caspase inhibitors using fluorometric methods. The assay utilizes synthetic peptide substrate YVAD-AFC (AFC, 7-amino-4-trifluoromethyl coumarin). Active caspase-1 cleaves the synthetic substrate to release free AFC which can then be quantified by fluorometry. Compounds to be screened can directly be added to the reaction and the level of inhibition of caspase-1 activity can be determined by comparison of the fluorescence intensity in samples with and without the testing inhibitors. The assay is simple, straightforward, and can be performed directly in microtiter plates. Each kit contains 100 units of active caspase-1, sufficient for screening 100 caspase inhibitor samples. Assay conditions have been optimized to obtain the maximal 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. 2X Reaction Buffer: 10 ml.
2. Caspase Substrate YVAD-AFC (1 mM): 0.5 ml.
3. DTT (1 M): 100 µl.
4. Active Caspase-1 (Lyophilized): 100 units.
5. Caspase Inhibitor, Z-VAD-FMK (2 mM): 10 µl.

B. Stability and storage

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

C. General Considerations & Reagent Preparations

- After thawing, store the 2X Reaction Buffer at 4℃. 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).
- Protect YVAD-AFC from light.
- Reconstitute the Active Caspase-1 in 550 µl 2X Reaction Buffer. Aliquote and immediately store at -70℃.

D. Protocol

1. Prepare testing sample in dH_2O to a final volume of 50 µl/well. Add 5 µl of Active Caspase-1. Mix well. Prepare a background control by omitting the Active Caspase-1 from the reaction mixture. Prepare a positive inhibition control by adding 1 µl of the Caspase-1 Inhibitor (provided with the kit) instead of your testing inhibitor.
2. Prepare a Master Mix for each assay containing the follows:
   45 µl 2X Reaction Buffer (containing 10 mM DTT) 5 µl
   1 mM YVAD-AFC substrate (50 µM final concentration)
3. Mix well and add 50 µl of the Master Mix to each well to start the reaction.
4. Incubate at 37℃ for 0.5-1 hour.
5. Read samples in a fluorescence plate reader equipped with a 400-nm excitation filter and 505-nm emission filter. Comparison of the fluorescence intensity of the testing samples with samples containing no inhibitors to determine the inhibition efficiency of the testing inhibitors.