MBL International Corporation

Caspase-10 Inhibitor Screening Kit

(Catalog #JM-K160-100; 100 assays; Store at -20°C)

I. Introduction:

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-10 Inhibitor Drug Screening Kit provides an effective means for screening caspase inhibitors using fluorometric methods. The assay utilizes synthetic peptide substrate AEVD-AFC (AFC, 7-amino-4-trifluoromethyl coumarin). Active caspase-10 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-10 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-10, sufficient for screening 100 caspase inhibitor samples. Assay conditions have been optimized to obtain the maximal activity.

II. Kit Contents:

Components	JM-K160-100 100 assays
2X Reaction Buffer	10 ml
Caspase Substrate AEVD-AFC (1 mM)	0.5 ml
DTT (1 M)	100 µl
Active Caspase-10 (Lyophilized)	100 units
Caspase-10 Inhibitor	10 µl

III. Caspase-10 Assay Protocol:

A. General Considerations & Reagent Preparations

- After thawing, store the 2X Reaction Buffer at 4°C. 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 AEVD-AFC from light.
- Reconstitute the Active Caspase-10 in 550 µl 2X Reaction Buffer. Aliquot and immediately store at –70°C.

B. Assay Procedure

1. Prepare testing sample in dH_2O to a final volume of 50 µl/well. Add 5 µl of Active Caspase-10. Mix well.

   Prepare a background control by omitting the Active Caspase-10 from the reaction mixture. Prepare a positive inhibition control by adding 1 µl of the Caspase-10 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 AEVD-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°C 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.

IV. Storage and Stability:

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

V. Related Products:

Apoptosis Detection Kits & Reagents
- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Additional Apoptosis Kits & Reagents

Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence
- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit