Caspase-8 Drug Screening Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K158
Description	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 PromoKine Caspase-8 Drug Screening Kit provides an effective means for screening caspase inhibitors using a fluorometric approach. The assay utilizes a synthetic peptide substrate IETD-AFC (AFC, 7-amino-4-trifluoromethyl coumarin). Active Caspase-8 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-8 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-8, sufficient for screening 100 caspase inhibitor samples. Assay conditions have been optimized to obtain the maximal activity.
Quantity	100 assays
Kit Components	Components – Quantity Active Caspase-8 (lyophilized) – 100 units 2x Reaction Buffer – 10 ml IETD-AFC (1 mM) – 0.5 ml DTT (1 M) – 100 µl Caspase Inhibitor, Z-VAD-FMK (2 mM) – 10 µl
Applications / Assay Protocol	A. General Consideration & Reagent Preparation 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 IETD-AFC from light. Reconstitute the Active Caspase-8 in 550 µl 2X Reaction Buffer. Aliquote 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-8. Mix well. Prepare a background control by omitting the Active Caspase-8 from the reaction mixture. Prepare a positive inhibition control by adding 1 µl of the Caspase-8 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 IETD-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.
Storage & Stability	Store kit at –20°C (Store Cell Lysis Buffer, 2X Reaction Buffer, and Dilution Buffer at 4°C after opening). All reagents are stable for at least 6 months.

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