Product Information

Caspase-8 Inhibitor Drug Screening Kit (Fluorometric)

I. Kit Contents:

Component	K2164-100 100 assays	Part Number
2X Reaction Buffer	10 ml	K2164-C-1
Caspase Substrate IETD-AFC (1 mM)	0.5 ml	K2164-C-2
DTT (1 M)	100 µl	K2164-C-3
Active Caspase-8 (Lyophilized)	100 units	K2164-C-4
Caspase Inhibitor, Z-VAD-FMK (2 mM)	10 µl	A1902

II. Introduction:

Caspases (Cysteine-dependent aspartate-directed proteases) are a family of cysteine proteases that play important roles in apoptosis, inflammation and necrosis. Sequential activation of caspases plays an important role in cell apoptosis. Inhibition of caspases can delay apoptosis, indicating a potential role in drug screening efforts. Caspase-8 is a caspase protein that involved in the programmed cell death induced by Fas and different apoptotic stimuli, and cleaves and activates Caspase-3. Caspase-8 was detected in the insoluble fraction of the affected brain region in Huntington disease patients, which suggested it plays a critical role in neurodegenerative diseases.

The Caspase-8 Inhibitor Drug Screening Kit (Fluorometric) provides a simple, fast and convenient way for screening of caspase-8 inhibitors based on fluorometric method. The synthetic peptide substrate IETD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin) emits blue light (λmax = 400 nm). While cleavage of IETD-AFC by active caspase-8, free AFC emits a yellow-green fluorescence (λmax = 505 nm) that can be quantified by a fluorecence microtiter plate reader or a fluorometer. Inhibitors can be directly added to the reaction and the efficacy of inhibition of caspase-8 activity is determined by comparison of the fluorescence intensity in samples without and with the testing inhibitors.

III. 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.

IV. Caspase-8 Assay Protocol:

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:

2X Reaction Buffer (containing 10 mM DTT)	45 µl
1 mM IETD-AFC substrate (50 µM final concentration)	5 µl

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.

V. 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.

General Troubleshooting Guide For Caspase Inhibitor Drug Screening Kits:

Problems	Cause	Solution
Assay not working	• Inactive Caspases due to incorrect reconstitution and storage • Use of degraded Caspase substrate • Plate read at incorrect wavelength • Old DTT used	• Reconstitute in reaction buffer, aliquot and store as described in the datasheet • Protect tube from direct light and store appropriately • Check the wavelength listed in the datasheet and the filter settings of the instrument • Always use freshly thawed DTT
High Background	• Increased amounts of components added due to incorrect pipetting • Use of substrate that has been exposed to light for extended periods	• Use calibrated pipettes • Store and handle substrate as indicated in the data sheet
Lower signal levels	• Incorrect setting of the equipment used to read samples • Allowing the reagents to sit for extended times on ice	• Refer to datasheet and use the recommended filter setting • Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	• Drugs tested at lower/ higher concentrations • Drugs prepared in a different buffer • Presence of interfering substance in the drug sample • Measured at incorrect wavelength • Drug samples contains interfering substances	• Refer literature and use appropriate concentrations; test several concentrations • Check if the components of the buffer could inhibit the reaction • Troubleshoot as needed • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit (run proper controls)
General issues	• Improperly thawed components • Incorrect incubation times or temperatures • Incorrect volumes used • Air bubbles formed in the well/tube • Substituting reagents from older kits/ lots • Use of a different 96-well plate	• Thaw all components completely and mix gently before use • Refer to datasheet & verify the correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly • Pipette gently against the wall of the well/tubes • Use fresh components from the same kit • Fluorescence: Black plates; Absorbance: Clear plates
Note: The most probable cause is listed under each section. Causes may overlap with other sections		

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Our promise

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