AssayGenie

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Caspase-3 Inhibitor Drug Screening Kit (Fluorometric) (BN00433)

(Catalog BN00433; 100 assays; Store kit 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-3 Inhibitor Drug Screening Kit provides an effective means for screening caspase inhibitors using fluorometric methods. The assay utilizes synthetic peptide substrate DEVD-AFC (AFC, 7-amino-4-trifluoromethyl coumarin). Active caspase-3 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-3 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-3, sufficient for screening 100 inhibitor samples. Assay conditions have been optimized to obtain the maximal activity.

II. Kit Contents:

Components	BN00433	Cap Code
2X Reaction Buffer	10 ml	NM
Caspase Substrate DEVD-AFC (1 mM)	0.5 ml	Brown
DTT (1 M)	100 µl	Blue
Active Caspase-3 (Lyophilized)	100 units	Orange
Caspase-3 Inhibitor	10 µl	Brown/Dot

III. Caspase-3 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 DEVD-AFC from light.
- Reconstitute the Active Caspase-3 in 550 µl 2X Reaction Buffer. Aliquot and immediately store at –70° C.
- We recommend using a flat bottom, opaque, white or black 96-well plate for enhanced sensitivity.

B. Assay Procedure

1. Prepare testing sample in dH_2O to a final volume of 50 µl/well. Add 5 µl of Active Caspase-3. Mix well.

   Prepare a background control by omitting the Active Caspase-3 from the reaction mixture. Prepare a positive inhibition control by adding 1 µl of the Caspase-3 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 DEVD-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.

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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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