



Caspase-9 Inhibitor Screening Kit

Catalog Number KA0763

100 assays

Version: 03

Intended for research use only

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Introduction

Background

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-9 Inhibitor Drug Screening Kit provides an effective means for screening caspase inhibitors using fluorometric methods. The assay utilizes synthetic peptide substrate LEHD-AFC (AFC, 7-amino-4-trifluoromethyl coumarin). Active caspase-9 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-9 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-9, sufficient for screening 100 caspase inhibitor samples. Assay conditions have been optimized to obtain the maximal activity.

General Information

Materials Supplied

List of component

Component	Amount
2X Reaction Buffer	10 mL
Caspase Substrate LEHD-AFC (1 mM)	0.5 mL
DTT (1 M)	100 μ L
Active Caspase-9 (Lyophilized)	100 units
Caspase Inhibitor, Z-VAD-FMK (2 mM)	10 μ L

Storage Instruction

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.

Assay Protocol

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 μ L of 1.0 M DTT stock per 1 mL of 2X Reaction Buffer).
- Protect LEHD-AFC from light.
- Reconstitute the Active Caspase-9 in 550 μ L 2X Reaction Buffer. Aliquote and immediately store at -70°C.

Assay Procedure

1. Prepare testing sample in dH₂O to a final volume of 50 μ L/well. Add 5 μ L of Active Caspase-9. Mix well. Prepare a background control by omitting the Active Caspase-9 from the reaction mixture. Prepare a positive inhibition control by adding 1 μ L of the Caspase-9 Inhibitor (provided with the kit) instead of your testing inhibitor.
2. Prepare a Master Mix for each assay containing the follows:
45 μ L 2X Reaction Buffer (containing 10 mM DTT)
5 μ L 1 mM LEHD-AFC substrate (50 μ M final concentration)
3. Mix well and add 50 μ L 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.

Resources

Trouble shooting

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> Inactive Caspases due to incorrect reconstitution and storage Use of degraded Caspase substrate Plate read at incorrect wavelength Old DTT used 	<ul style="list-style-type: none"> 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
High Background	<ul style="list-style-type: none"> Increased amounts of components added due to incorrect pipetting Use of substrate that has been exposed to light for extended periods 	<ul style="list-style-type: none"> Use calibrated pipettes Store and handle substrate as indicated in the data sheet
Lower signal levels	<ul style="list-style-type: none"> Incorrect setting of the equipment used to read samples Allowing the reagents to sit for extended times on ice 	<ul style="list-style-type: none"> Refer to datasheet and use the recommended filter setting Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> 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)

<p>General issues</p>	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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
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Note: The most probable cause is listed under each section. Causes may overlap with other sections.