ab102497

Caspase 9 Inhibitor Drug Detection Kit

Instructions for Use

For the rapid, sensitive and accurate detection of Caspase 9 inhibition by various compounds

This product is for research use only and is not intended for diagnostic use.

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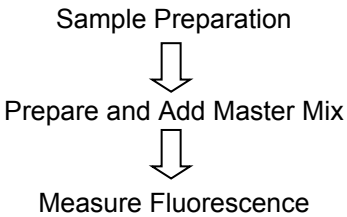
1. Overview

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.

Abcam's Caspase 9 Inhibitor Drug Detection 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 be directly 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

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

* Store kit at -20°C. All components are stable for 6 months under proper storage conditions.

Protect LEHD-AFC from light.

REACTION BUFFER: 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).

ACTIVE CASPASE 9: Reconstitute in 550 µl 2X Reaction Buffer. Aliquot and immediately store at -80°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Prepare test samples in dH_2O up to a final volume of 50 µl/well. Add 5 µl 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 µl of the Caspase 9 Inhibitor (provided with the kit) instead of your testing inhibitor.

Note: This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.

2. Prepare a Master Mix for each assay containing the following:

2X Reaction Buffer (containing 10 mM DTT)	45 µl
1 mM LEHD-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. Incubate at 37°C for 0.5-1 hour.

4. Read samples in a fluorescence plate reader equipped with a 400-nm excitation filter and 505-nm emission filter.

Compare the fluorescence intensity of the test samples with samples containing no inhibitors to determine the inhibition efficiency of the testing inhibitors.

5. Factors to consider for caspase activity assays

Three major factors need to be taken into account when using caspase activity assays:

1. The substrate in a particular assay is not necessarily specific to a particular caspase.

   Cleavage specificities overlap so reliance on a single substrate/assay is not recommended. Other assays, such as Western blot or use of fluorescent substrates e.g. FRET assays should be used in combination with caspase activity assays.

2. The expression and abundance of each caspase in a particular cell type and cell line will vary.

3. As the activation and cleavage of caspases in the cascade will change over time, you should consider when particular caspase will be at its peak concentration e.g. after 3 hours, after 20 hours etc.

The table below shows the known cross-reactivities with other caspases.

Classification of caspases based on synthetic substrate preference, does not reflect the real caspase substrate preference *in vivo* and may provide inaccurate information for discriminating amongst caspase activities. Thus, caution is advised in applying the intrinsic tetrapeptide preferences to predict the targets of individual caspases.

Apoptotic Initiator Caspases

Caspase	Cleavage motif	Inhibitor motif	Cross-reactivity with other caspase:									
			1	2	3	4	5	6	7	8	9	10
Caspase 2	VDVAD				Y				Y			
Caspase 8	IETD	IETD, LETD			Y			Y				Y
Caspase 9	LEHD				Y			Y		Y		Y
Caspase 10	AEVD				Y				Y	Y?		

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Reaction buffer at wrong temperature	Assay buffer must be at 4°C
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
General Issues	Improperly thawed components	Thaw all components completely and mix gently before use
	Incorrect incubation times or temperatures	Refer to datasheet & verify the correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Air bubbles formed in the well/tube	Pipette gently against the wall of the well/tubes
	Substituting reagents from older kits/ lots	Use fresh components from the same kit

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp