ab102488

Caspase Family Colorimetric Substrate Kit II Plus

Instructions for Use

For the rapid, sensitive and accurate measurement of Caspase activity in cell culture

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

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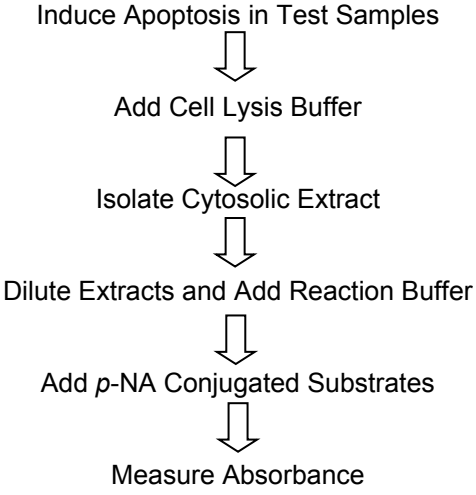
1. Overview

Abcam's Caspase Family Colorimetric Substrate Kit II Plus provides ready-to-use colorimetric substrates for members of caspase family proteases. All substrates are provided in liquid ready-to-use form.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
YVAD-*p*-NA	125 µL
Ac-VDVAD-*p*-NA	125 µL
DEVD-*p*-NA	125 µL
Caspase 4 Substrate	125 µL
Ac-WEHD-*p*-NA	125 µL
Ac-VEID-*p*-NA	125 µL
Caspase 8 Substrate	125 µL
Caspase 9 Substrate	125 µL
AEVD-*p*-NA	125 µL
Lysis Buffer IV	100 mL
Dilution Buffer II	200 mL
Caspase 2X Reaction Buffer	20 mL

Item	Quantity
DTT I	400 µL

PLEASE NOTE: The components in the table were previously labeled under the following names:

- YVAD-pNA was previously labelled as 4 mM Caspase 1 Substrate & Ac-YVAD-p-NA
- Ac-VDVAD-p-NA as 4 mM Caspase 2 Substrate
- DEVD-p-NA as 4 mM Caspase 3 Substrate & Ac-DEVD-p-NA
- Caspase 4 Substrate as Ac-LEVD-p-NA
- Ac-WEHD-p-NA as 4 mM Caspase 5 Substrate
- Ac-VEID-p-NA as 4 mM Caspase 6 Substrate
- Caspase 8 Substrate as Ac-IETD-p-NA
- Caspase 9 Substrate as Ac-LEHD-p-NA
- AEVD-pNA as 4 mM Caspase 10 Substrate & Ac-AEVD-p-NA
- Lysis Buffer IV as Cell Lysis Buffer
- Dilution Buffer II as Dilution Buffer
- Caspase 2X Reaction Buffer as 2X Reaction Buffer
- DTT I as 1 M DTT

The compositions have not changed.

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

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader or spectrophotometer
- 96 well plate
- Micro-quartz and regular cuvettes
- Orbital shaker

4. Assay Protocol

1. Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture *without* treatment.

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

2. Count cells and pellet 1-5 x 10^6 cells.

3. Re-suspend in 50 µl of chilled Lysis Buffer IV and incubate on ice for 10 min.

4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).

5. Transfer supernatant to a fresh tube and keep on ice.

6. Assay protein concentration.

7. Dilute 100-300 µg protein to 50 µl Lysis Buffer IV for each assay.

8. Add 50 µl of Caspase 2X Reaction Buffer (containing 10 mM DTT I) to each sample.

9. Add 5 µl of the 4 mM *p*-NA conjugated substrates (200 µM final conc.) into each tube individually and incubate at 37°C for 1-2 hours.

10. Read samples at 400-nm or 405-nm in a microtiter plate reader, or spectrophotometer using a 100-µl micro quartz cuvette, or dilute sample to 1 ml with Dilution Buffer II and using regular cuvette (Dilution of the samples proportionally decreases the reading).

Fold-increase in caspase activity can be determined by comparing the results of induced samples with the level of the un-induced control.

Note:

Background reading from cell lysates and buffers should be subtracted from the readings of both treated and the untreated samples before calculating fold increase in caspase activity.

5. Troubleshooting

Problem	Reason	Solution
Assay not working	Cells did not lyse completely	Re-suspend the cell pellet in the lysis buffer and incubate as described in the datasheet
	Experiment was not performed at optimal time after apoptosis induction	Perform a time-course induction experiment for apoptosis
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Old DTT I used	Always use freshly thawed DTT I in the Lysis Buffer IV
High Background	Increased amount of cell lysate used	Refer to datasheet and use the suggested cell number to prepare lysates
	Increased amounts of components added due to incorrect pipetting	Use calibrated pipettes
	Incubation of cell samples for extended periods	Refer to datasheet and incubate for exact times
	Use of expired kit or improperly stored reagents	Always check the expiry date and store the individual components appropriately
	Contaminated cells	Check for bacteria/ yeast/ mycoplasma contamination

Problem	Reason	Solution
Lower signal levels	Cells did not initiate apoptosis	Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	Very few cells used for analysis	Refer to datasheet for appropriate cell number
	Use of samples stored for a long time	Use fresh samples or aliquot and store and use within one month for the assay
	Incorrect setting of the equipment used to read samples	Refer to datasheet and use the recommended filter setting
	Allowing the reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	Uneven number of cells seeded in the wells	Seed only equal number of healthy cells (correct passage number)
	Samples prepared in a different buffer	Use the Lysis Buffer IV provided in the kit
	Adherent cells dislodged and lost at the time of experiment	Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters
	Cell/ tissue samples were not completely homogenized	Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples, if needed to use multiple times
	Presence of interfering substance in the sample	Troubleshoot as needed
	Use of old or inappropriately stored samples	Use fresh samples or store at correct temperatures until use

Unexpected results	Measured at incorrect wavelength	Check the equipment and the filter setting
	Cell samples contain interfering substances	Troubleshoot if it interferes with the kit (run proper controls)
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
	Use of a different 96-well plate	Fluorescence: Black plates; Absorbance: Clear plates

Technical Support

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