ab102487

Caspase Family Fluorometric 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.

Version 2 Last updated 11 February 2019

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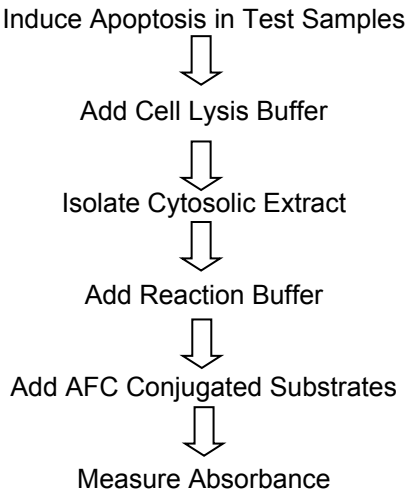
1. Overview

Abcam's Caspase Family Fluorometric Substrate Kit II Plus provides ready-to-use fluorometric substrates for assaying activities of 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
1 mM Caspase 1 Substrate (Ac-YVAD-AFC)	125 µL
1 mM Caspase 2 Substrate (Ac-VDVAD-AFC)	125 µL
1 mM Caspase 3 Substrate (Ac-DEVD-AFC)	125 µL
1 mM Caspase 4 Substrate (Ac-LEVD-AFC)	125 µL
1 mM Caspase 5 Substrate (Ac-WEHD-AFC)	125 µL
1 mM Caspase 6 Substrate (Ac-VEID-AFC)	125 µL
1 mM Caspase 8 Substrate (Ac-IETD-AFC)	125 µL
1 mM Caspase 9 Substrate (Ac-LEHD-AFC)	125 µL
1 mM Caspase 10 Substrate (Ac-AEVD-AFC)	125 µL

Item	Quantity
Cell Lysis Buffer	100 mL
2X Reaction Buffer	20 mL
1 M DTT	0.4 mL

* Store kit at -20°C. Substrates are dissolved in DMSO. All reagents are stable for 6-12 months under proper storage conditions.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorometric microplate reader or fluorometer
- 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 or use 50-200 µg cell lysates if protein concentration has been measured.

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

4. Add 50 µl of 2X Reaction Buffer and 1 µl DTT to each sample.

5. Add 5 µl of the 1 mM AFC conjugated substrates (50 µM final conc.) into each tube individually and incubate at 37°C for 1-2 hours.

6. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. You may perform the entire assay directly in a 96-well plate.

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

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 used	Always use freshly thawed DTT in the cell lysis buffer
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

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 cell lysis buffer 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

Problem	Reason	Solution
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

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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