Caspase-Family Fluorometric Substrate Set

CATALOG #: BN00170

LOT #: _____

STORAGE CONDITIONS: Store at –20° C.

SHELF LIFE: 1 year under proper storage conditions

DESCRIPTION:

Fluorometric substrates for assaying activities of members of caspase family proteases. All substrates are provided in liquid ready-to-use form.

Kit CONTENTS: Each of the following substrates is dissolved in DMSO

Concentration	Description	Volume	Part Number
1 mM	Caspase-1 Substrate, Ac-YVAD-AFC	125 µl	BN00170-3
1 mM	Caspase-2 Substrate, Ac-VDVAD-AFC	125 µl	BN00170-3
1 mM	Caspase-3 Substrate, Ac-DEVD-AFC	125 µl	BN00170-3
1 mM	Caspase-5 Substrate, Ac-WEHD-AFC	125 µl	BN00170-3
1 mM	Caspase-6 Substrate, Ac-VEID-AFC	125 µl	BN00170-3
1 mM	Caspase-8 Substrate, Ac-IETD-AFC	125 µl	BN00170-3
1 mM	Caspase-9 Substrate, Ac-LEHD-AFC	125 µl	BN00170-3

ASSAY PROCEDURE:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
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. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat. #BN01067).
4. Incubate cells on ice for 10 minutes.
5. Add 50 µl of 2X Reaction Buffer (Cat. # BN01068) containing 10 mM DTT to each sample.
6. 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 hour.
7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may perform the entire assay directly in a 96-well plate.

   Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.

FOR RESEARCH USE ONLY! Not to be used on humans.

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GENERAL TROUBLESHOOTING GUIDE FOR CASPASE COLORIMETRIC AND FLUOROMETRIC KITS:

Problems	Cause	Solution
Assay not working	• Cells did not lyse completely	• Resuspend 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	• Check the wavelength listed in the datasheet and the filter settings of the instrument
	• 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
Unanticipated 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
Note: The most probable cause is listed under each section. Causes may overlap with other sections.		

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