Product Information

Caspase Colorimetric Substrate Set

I. Kit Contents:

Description	Concentration	7 x 25 assays	Part Number
Caspase-1 Substrate, Ac-YVAD-pNA	4 mM	125 µl	K2203-C-1
Caspase-2 Substrate, Ac-VDVAD-pNA	4 mM	125 µl	K2203-C-2
Caspase-3 Substrate, Ac-DEVD-pNA	4 mM	125 µl	K2203-C-3
Caspase-5 Substrate, Ac-WEHD-pNA	4 mM	125 µl	K2203-C-4
Caspase-6 Substrate, Ac-VEID-pNA	4 mM	125 µl	K2203-C-5
Caspase-8 Substrate, Ac-IETD-pNA	4 mM	125 µl	K2203-C-6
Caspase-9 Substrate, Ac-LEHD-pNA	4 mM	125 µl	K2203-C-7

II. Introduction:

Caspases (Cysteine-dependent aspartate-directed proteases) are a family of cysteine proteases that play important roles in apoptosis, inflammation and necrosis. Sequential activation of caspases plays a critical role in cell apoptosis. Caspase-1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are members of caspases family. Caspase-1, 4 and 5 are involved in T-cell maturation. Caspase-2, 8, 9 and 10 are initiator caspases that cleave inactive pro-forms of effector caspases into active caspases. Caspase-3, 6 and 7 are effector caspases that cleave other protein substrates within the cell to trigger the apoptotic process.

Caspase Colorimetric Substrate Set is ready-to-use for members of caspase family proteases. All substrates were provided in liquid ready-to-use form.

III. 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.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant to a fresh tube and assay protein Concentration.
6. Dilute 50 - 200 µg protein to 50 µl Cell Lysis Buffer for each assay.
7. Add 50 µl of 2X Reaction Buffer containing 10 mM DTT to each sample.
8. Add 5 µl of the 4 mM pNA conjugated substrates (200 µM final conc.) into each tube individually and incubate at 37℃ for 1-2 hour.
9. Read samples at 400 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 and using regular cuvette (note: Dilution of the samples proportionally decreases the reading).

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

Note: Background reading from cell lysates and buffers should be subtracted from the readings of both induced and the uninduced samples before calculating fold increase in caspase activity.

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

	• Experiment was not performed at optimal time after apoptosis induction • Plate read at incorrect wavelength • Old DTT used	described in the datasheet • Perform a time-course induction experiment for apoptosis • Check the wavelength listed in the datasheet and the filter settings of the instrument • Always use freshly thawed DTT in the cell lysis buffer
High Background	• Increased amount of cell lysate used • Increased amounts of components added due to incorrect pipetting • Incubation of cell samples for extended periods • Use of expired kit or improperly stored reagents • Contaminated cells	• Refer to datasheet and use the suggested cell number to prepare lysates • Use calibrated pipettes • Refer to datasheet and incubate for exact times • Always check the expiry date and store the individual components appropriately • Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	• Cells did not initiate apoptosis • Very few cells used for analysis • Use of samples stored for a long time • Incorrect setting of the equipment used to read samples • Allowing the reagents to sit for extended times on ice	• Determine the time-point for initiation of apoptosis after induction (time-course experiment) • Refer to datasheet for appropriate cell number • Use fresh samples or aliquot and store and use within one month for the assay • Refer to datasheet and use the recommended filter setting • Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	• Uneven number of cells seeded in the wells • Samples prepared in a different buffer • Adherent cells dislodged and lost at the time of experiment • Cell/ tissue samples were not completely homogenized • Samples used after multiple freeze-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Seed only equal number of healthy cells (correct passage number) • Use the cell lysis buffer provided in the kit • Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters • Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope • Aliquot and freeze samples, if needed to use multiple times • Troubleshoot as needed • Use fresh samples or store at correct temperatures until use
Unanticipated results	• Measured at incorrect wavelength • Cell samples contain interfering substances	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit (run proper controls)
General issues	• 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	• 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
Note# The most probable cause is listed under each section. Causes may overlap with other sections.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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