Product Information

Caspase-9 Fluorometric Assay Kit

I. Kit Contents:

Component	K2018-25 25 assays	K2018-100 100 assays	K2018-200 200 assays	K2018-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K2018-C-1
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K2018-C-2
LEHD-AFC (1 mM)	125 µl	500 µl	2 x 0.5 ml	2 x 1 ml	K2018-C-3
DTT (1 M)	100 µl	400 µl	400 µl	400 µl	K2018-C-4

II. Introduction:

Cysteine-dependent aspartate-directed proteases (Caspases) are a family of cysteine proteases that play important roles in apoptosis, necrosis, and inflammation. Sequential activation of caspases plays an important role in cell apoptosis. Caspase-9 is a member of the caspase family. Caspase-9, as well as caspase-2, -8 and -10, is an initiator caspase. Caspase-9 is involved in the mitochondrial death pathway and is activated during apoptosis. The activation of JNK/SAPK stress signaling pathways induces the release of cytochrome c from mitochondria and activation of apaf-1, which then cleaves caspase-9 into the active form. The active Caspase-9 cleaves and activates Caspase-3 and Caspase-7, which then cleave poly ADP ribose polymerase.

Caspase-9 Fluorometric Assay Kit provides a convenient and simple way for detecting the LEHD-dependent caspase activity. LEHD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin) emits blue light (λmax = 400 nm); while cleavage of the substrate LEHD-AFC by Caspase-9 or related caspases, free AFC emits a yellow-green fluorescence (λmax = 505 nm), which can be quantified by using a fluorecence microtiter plate reader or a fluorometer. Comparison of the fluorescence of free AFC from an apoptotic sample with an uninduced control determines the fold increase in Caspase-9 activity.

III. Caspase-9 Assay Protocol:

A. General Considerations

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).

Protect LEHD-AFC from light.

B. 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. Incubate cells on ice for 10 minutes.

4. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 1 mM LEHD-AFC substrate (50 µM final concentration) and incubate at 37℃ for 1-2 hour.

5. 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 also perform the entire assay directly in a 96-well plate.

Fold-increase in Caspase-9 activity can be determined by comparing these results with the level of the uninduced control.

IV. Storage and Stability:

Store kit at -20℃ (Store Cell Lysis Buffer and 2X Reaction Buffer at 4℃ after opening). All reagents are stable for 1 year under proper storage conditions.

General Troubleshooting Guide for Caspase Colorimetric and Fluorometric Kits:

Problems	Cause	Solution
Assay not working	• Cells did not lyse completely • Experiment was not performed at optimal time after apoptosis induction • Plate read at incorrect wavelength • Old DTT used	• Resuspend the cell pellet in the lysis buffer and incubate as 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	• Thaw all components completely and mix gently before use

	• 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	• 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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