Product Information

Caspase-4 Fluorometric Assay Kit

I. Kit Contents:

Component	K2198-25 25 assays	K2198-100 100 assays	K2198-200 200 assays	K2198-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K2198-C-1
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K2198-C-2
LEHD-AFC (1 mM)	125 µl	500 µl	2 x 0.5 ml	2 x 1 ml	K2198-C-3
DTT (1 M)	100 µl	400 µl	400 µl	400 µl	K2198-C-4

II. Introduction:

Caspase-4 belongs to the Caspase-family of cysteine proteases. Caspase-4 is an enzyme that proteolytically cleaves other proteins at an aspartic acid residue. Caspase-4 exists in cells as an inactive proenzyme. It is matured by proteolysis. The active Caspase-4 is a heterotetramer contain two large and two small subunits. The function of Caspase 4 is believed to be an inflammatory caspase, together with Caspase-1, Caspase-5, with a role in the immune system.

Apoptosis in mammalian cells is caused by activation of ICE-family proteases/caspases. The Caspase-4 Fluorometric Assay Kit provides a fast and simple means to assay the activity of caspases that recognize the sequence LEVD. The assay is according to detection of cleavage of substrate LEVD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). LEVD-AFC emits blue light (400 nm); free AFC emits a yellow-green fluorescence (505 nm) upon cleavage of the substrate by Caspase-4 or other related Caspases. Using a fluorometer or a fluorecence microtiter plate reader can quantify the fluorescence. Comparison of the fluorescence of AFC from an apoptotic sample with an un-induced control allows determination of the fold increase in LEVD-dependent Caspase activity.

III. Caspase-5 Assay Protocol:

A. Reagent Preparation

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).

B. Assay Procedure

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.

2. Count cells and pellet 2 - 5 x 10^6 cells or use 100-200 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 LEVD-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 in a 96-well plate.

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

IV. Storage and Stability:

Store kit at -20℃ (Store Cell Lysis Buffer & 2X Reaction Buffer at 4℃ after opening). Protect LEVD-AFC from light. All reagents are stable for 6 months 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 • Incorrect incubation times or temperatures • Incorrect volumes used • Air bubbles formed in the well/tube	• 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

	• Substituting reagents from older kits/ lots • Use of a different 96-well plate	• 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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