Product Information

Caspase-2 Colorimetric Assay Kit

I. Kit Contents:

Component	K2017-25 25 assays	K2017-100 100 assays	K2017-200 200 assays	K2017-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K2017-C-1
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K2017-C-2
VDVAD-pNA (4 mM)	125 µl	500 µl	2 x 0.5 ml	2 x 1 ml	K2017-C-3
DTT (1 M)	100 µl	400 µl	400 µl	400 µl	K2017-C-4
Dilution Buffer	25 ml	100 ml	200 ml	400 ml	K2017-C-5

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-2 is a member of the caspase family. Caspase-2, as well as caspase-8, -9 and -10, is an initiator caspase. Caspase-2 has a strict requirement for an Asp residue for proteolytic activity and has a preferred cleavage sequence of Val-Asp-Val-Ala-Asp- (VDVAD).

Caspase-2 Colorimetric Assay Kit provides a convenient and simple way for detecting the VDVAD-dependent caspase activity. When cleavage of VDVAD from the labeled substrate VDVAD-p-nitroaniline (VDVAD-pNA) by Caspase-2 or related caspases, the free pNA light emission can be quantified by using a microtiter plate reader or a spectrophotometer at 405 or 400 nm. Comparison of the absorbance of free pNA from an apoptotic sample with an uninduced control determines the fold increase in Caspase-2 activity.

III. Caspase-2 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).

After thawing, store the Cell Lysis Buffer and Dilution Buffer at 4℃. All kit reagents are stable for 6 months under proper storage conditions.

Protect VDVAD-pNA 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 2 - 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 (cytosolic extract) to a fresh tube and put on ice.
6. Assay protein concentration.
7. Dilute 100 - 200 µg cytosolic extract to 50 µl Cell Lysis Buffer for each assay.
8. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 4 mM VDVAD-pNA substrate (200 µM final conc.) 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 (Sigma), or dilute sample to 1 ml with Dilution 10. Buffer and using regular cuvette (note: Dilution of the samples proportionally decreases the reading).

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

Fold-increase in Caspase-2 activity can be determined by comparing the results of treated samples with the level of the uninduced control.

IV. 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-2 activity.

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 • 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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