Caspase-3 Colorimetric Assay Kit

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-3 is a caspase protein that cleaves and activates Caspases-6 and -7, and is processed and activated by Caspases-8, -9, and 10. Caspase-3 is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with Alzheimer's disease. Caspase-3 recognizes tetra-peptide sequences D-x-x-D and hydrolyzes peptide bonds after aspartic acid residues.

Caspase-3 Colorimetric Assay Kit provides a convenient and simple way for detecting the DEVD-dependent caspase activity. When cleavage of DEVD from the labeled substrate DEVD-p-nitroaniline (DEVD-pNA) by Caspase-3 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-3 activity.

Components and Storage

Components	K2008-25 25 assays	K2008-100 100 assays	K2008-200 200 assays	K2008-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K2008-C-1
2 X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K2008-C-2
DEVD-pNA (4 mM)	125 µl	500 µl	2 x 0.5 ml	2 x 1 ml	K2008-C-3
DTT (1 M)	100 µl	400 µl	400 µl	400 µl	K2008-C-4
Store at -20°C.					

Protocol

1. General Considerations

Aliquot enough 2 X Reaction Buffer for the number of assays to be performed. Add DTT to the 2 X Reaction Buffer immediately before use (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2 X Reaction Buffer). Protect DEVD-pNA from light.

2. 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 20 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 for immediate assay or aliquot and store at -80°C for future use.

(6) Assay protein concentration.

(7) Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample.

(8) Add 5 µl of the 4 mM DEVD-pNA substrate (200 µM final conc.) and incubate at 37°C 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). Fold-increase in CPP32 activity can be determined by comparing these results with the level of the uninduced control.

Notes: 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 CPP32 activity.

Notes

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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