Caspase-6 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-6 is a member of the caspase family. Caspase-6 is processed by caspases 7, 8 and 10, and can also undergo self-processing. Caspase-6 acts as a downstream enzyme in the caspase activation cascade and interacts with Caspase 8.

Caspase-6 Colorimetric Assay Kit provides a convenient and simple way for detecting the VEID-dependent caspase activity. When cleavage of VEID from the labeled substrate VEID-p-nitroaniline (VEID-pNA) by Caspase-6 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-6 activity.

Components and Storage

Components	K2015-25 25 assays	K2015-100 100 assays	K2015-200 200 assays	K2015-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K2015-C-1
2 X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K2015-C-2
VEID-pNA (4 mM)	125 µl	500 µl	2 x 0.5 ml	2 x 1 ml	K2015-C-3
DTT (1 M)	100 µl	400 µl	400 µl	400 µl	K2015-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 VEID-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 2-5 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 VEID-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 caspase-6 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 caspase-6 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 • 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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