

Caspase-3 Fluorometric 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 Fluorometric Assay Kit provides a convenient and simple way for detecting the DEVD-dependent caspase activity. DEVD-AFC (AFC:7-amino-4-trifluoromethyl coumarin) emits blue light ($\lambda_{max} = 400 \text{ nm}$); while cleavage of DEVD-AFC by Caspase-3 or related caspases, free AFC emits a yellow-green fluorescence ($\lambda_{max} = 505 \text{ nm}$), which can be quantified by using a fluorescence microtiter plate reader or a fluorometer. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control determines the fold increase in Caspase-3 activity.

Components and Storage

Components	K2007-25 25 assays	K2007-100 100 assays	K2007-200 200 assays	K2007-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K2007-C-1
2 X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K2007-C-2
DEVD-AFC (1 mM)	125 μl	500 μl	2 x 0.5 ml	2 x 1 ml	K2007-C-3
DTT (1 M)	100 μl	400 μl	400 μl	400 μl	K2007-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). After thawing, store the Cell Lysis Buffer and 2X Reaction Buffer at 4°C.

Protect DEVD-AFC 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 $1-5 \times 10^6$ cells or use 20-200 μg cell lysates (depending on the apoptosis level).
- (3) Resuspend cells in 50 μl of chilled Cell Lysis Buffer.
- (4) Incubate cells on ice for 10 minutes.
- (5) Add 50 μl of 2X Reaction Buffer (containing 10 mM DTT) to each sample.
- (6) Add 5 μl of the 1 mM DEVD-AFC substrate (50 μM final concentration) and incubate at 37°C for 1-2 hour.
- (7) 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.

Tips:

- (1) For tissue samples, tissue can be homogenized in Lysis Buffer (for 1X volume of tissue, add 3X volume of lysis buffer) to generate tissue lysate, then follow the kit procedure.
- (2) Tissue and cell lysates can be kept frozen at -80°C for up to 2 months without significant loss of activity.

Notes

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Cells did not lyse completely • Experiment was not performed at optimal time after apoptosis induction • Plate read at incorrect wavelength • Old DTT used 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Cell samples contain interfering substances 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit (run proper controls)

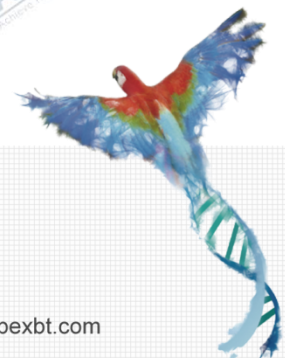
results

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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