

Caspase-9 Fluorometric Assay Kit

(Catalog #ALX-850-224; 25 tests Store kit at -20°C)

I. Introduction:

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **Caspase-9 Fluorometric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence LEHD. The assay is based on detection of cleavage of substrate LEHD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). LEHD-AFC emits blue light ($\lambda_{max} = 400 \text{ nm}$); upon cleavage of the substrate by caspase-9 or related caspases, free AFC emits a yellow-green fluorescence ($\lambda_{max} = 505 \text{ nm}$), which can be quantified using a fluorometer or fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase-9 activity.

II. Kit Contents:

Components	ALX-850-224-KI01	Part Number
	25 tests	
Cell Lysis Buffer	25ml	ALX-K118-25-1
2X Reaction Buffer	2ml	ALX-K118-25-2
LEHD-AFC (1mM)	125 μ l	ALX-K118-25-3
DTT (1M)	100 μ l	ALX-K118-25-4

III. Caspase-9 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 (10mM final concentration: add 10 μ l of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).
- Protect LEHD-AFC 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 $1-5 \times 10^6$ cells or use 50-200 μ g cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 μ l of chilled Cell Lysis Buffer. Incubate cells on ice for 10 minutes.
4. Add 50 μ l of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 μ l of the 1 mM LEHD-AFC substrate (50 μ M final concentration) and incubate at 37°C 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 directly in a 96-well plate.
6. Fold-increase in Caspase-9 activity can be determined by comparing these results with the level of the uninduced control.

IV. Storage and Stability:

Store kit at -20°C (Store Cell Lysis Buffer and 2X Reaction Buffer at 4°C after opening). All reagents are stable for 1 year under proper storage conditions.

V. Related Products:

Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Apoptosis siRNA Vectors

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Fluorometric & Colorimetric Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP & PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays

- siRNA Expression Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit
- β -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

GENERAL TROUBLESHOOTING GUIDE FOR CASPASE COLORIMETRIC AND FLUOROMETRIC KITS

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 results	<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)
General issues	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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.