

# Caspase-4 Fluorometric Assay Kit

(Catalog #K126-25, -100, -200, -400; Store kit at -20°C)

**I. Introduction:**

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

**II. Kit Contents:**

Components	K126-25	K126-100	K126-200	K126-400	Part Number
	25 assays	100 assays	200 assays	400 assays	
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K126-XX(X)-1
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K126-XX(X)-2
LEVD-AFC (1 mM)	125 $\mu\text{l}$	0.5 ml	2 x 0.5 ml	2 x 1 ml	K126-XX(X)-3
DTT (1 M)	100 $\mu\text{l}$	0.4 ml	0.4 ml	0.4 ml	K126-XX(X)-4

**III. Caspase-4 Assay Protocol:**

**A. Reagent Preparation**

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  $\mu\text{l}$  of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).

**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<sup>6</sup> cells or use 100-200  $\mu\text{g}$  cell lysates if protein concentration has been measured.
3. Resuspend cells in 50  $\mu\text{l}$  of chilled Cell Lysis Buffer. Incubate cells on ice for 10 minutes.
4. Add 50  $\mu\text{l}$  of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5  $\mu\text{l}$  of the 1 mM LEVD-AFC substrate (50  $\mu\text{M}$  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 in a 96-well plate.

Fold-increase in LEVD-dependent caspase activity can be determined by comparing the results of induced samples with the level of the uninduced control.

**IV. Storage and Stability:**

- Store kit at -20°C (Store Cell Lysis Buffer & 2X Reaction Buffer at 4°C after opening). Protect LEVD-AFC from light.
- All reagents are stable for 6 months 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 Set
- 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
- 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
- $\beta$  -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

Growth Factors and Cytokine

**FOR RESEARCH USE ONLY! Not to be used on humans.**

## GENERAL TROUBLESHOOTING GUIDE FOR CASPASE COLORIMETRIC AND FLUOROMETRIC KITS:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Cells did not lyse completely</li> <li>• Experiment was not performed at optimal time after apoptosis induction</li> <li>• Plate read at incorrect wavelength</li> <li>• Old DTT used</li> </ul>	<ul style="list-style-type: none"> <li>• Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet</li> <li>• Perform a time-course induction experiment for apoptosis</li> <li>• Check the wavelength listed in the datasheet and the filter settings of the instrument</li> <li>• Always use freshly thawed DTT in the cell lysis buffer</li> </ul>
High Background	<ul style="list-style-type: none"> <li>• Increased amount of cell lysate used</li> <li>• Increased amounts of components added due to incorrect pipetting</li> <li>• Incubation of cell samples for extended periods</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Contaminated cells</li> </ul>	<ul style="list-style-type: none"> <li>• Refer to datasheet and use the suggested cell number to prepare lysates</li> <li>• Use calibrated pipettes</li> <li>• Refer to datasheet and incubate for exact times</li> <li>• Always check the expiry date and store the individual components appropriately</li> <li>• Check for bacteria/ yeast/ mycoplasma contamination</li> </ul>
Lower signal levels	<ul style="list-style-type: none"> <li>• Cells did not initiate apoptosis</li> <li>• Very few cells used for analysis</li> <li>• Use of samples stored for a long time</li> <li>• Incorrect setting of the equipment used to read samples</li> <li>• Allowing the reagents to sit for extended times on ice</li> </ul>	<ul style="list-style-type: none"> <li>• Determine the time-point for initiation of apoptosis after induction (time-course experiment)</li> <li>• Refer to datasheet for appropriate cell number</li> <li>• Use fresh samples or aliquot and store and use within one month for the assay</li> <li>• Refer to datasheet and use the recommended filter setting</li> <li>• Always thaw and prepare fresh reaction mix before use</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Uneven number of cells seeded in the wells</li> <li>• Samples prepared in a different buffer</li> <li>• Adherent cells dislodged and lost at the time of experiment</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple freeze-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Seed only equal number of healthy cells (correct passage number)</li> <li>• Use the cell lysis buffer provided in the kit</li> <li>• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope</li> <li>• Aliquot and freeze samples, if needed to use multiple times</li> <li>• Troubleshoot as needed</li> <li>• Use fresh samples or store at correct temperatures until use</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Cell samples contain interfering substances</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit (run proper controls)</li> </ul>
General issues	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> <li>• Air bubbles formed in the well/tube</li> <li>• Substituting reagents from older kits/ lots</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Refer to datasheet &amp; verify the correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> <li>• Pipette gently against the wall of the well/tubes</li> <li>• Use fresh components from the same kit</li> <li>• Fluorescence: Black plates; Absorbance: Clear plates</li> </ul>

**Note#** The most probable cause is listed under each section. Causes may overlap with other sections.