

## Caspase-Family Fluorometric Substrate Set II

**CATALOG #:** K133-9-25  
**LOT #:** \_\_\_\_\_  
**STORAGE CONDITIONS:** Store at -20° C.  
**SHELF LIFE:** 1 year under proper storage conditions

**DESCRIPTION:**

Fluorometric substrates for assaying activities of members of caspase family proteases. All substrates are provided in liquid ready-to-use form.

**Kit CONTENTS:** Each of the following substrates is dissolved in DMSO

Concentration	Description	Volume	Part Number
1 mM	Caspase-1 Substrate, Ac-YVAD-AFC	125 µl	K110-25-3
1 mM	Caspase-2 Substrate, Ac-VDVAD-AFC	125 µl	K116-25-3
1 mM	Caspase-3 Substrate, Ac-DEVD-AFC	125 µl	K105-25-3
1 mM	Caspase-4 Substrate, Ac-LEVD-AFC	125 µl	K126-25-3
1 mM	Caspase-5 Substrate, Ac-WEHD-AFC	125 µl	K122-25-3
1 mM	Caspase-6 Substrate, Ac-VEID-AFC	125 µl	K114-25-3
1 mM	Caspase-8 Substrate, Ac-IETD-AFC	125 µl	K112-25-3
1 mM	Caspase-9 Substrate, Ac-LEHD-AFC	125 µl	K118-25-3
1 mM	Caspase-10 Substrate, Ac-AEVD-AFC	125 µl	K124-25-3

**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<sup>6</sup> cells or use 50-200 µg cell lysates if protein concentration has been measured.
  3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# 1067-100).
  4. Incubate cells on ice for 10 minutes.
  5. Add 50 µl of 2X Reaction Buffer (Cat. # 1068-20) containing 10 mM DTT (Cat.# 1201-1) to each sample.
  6. Add 5 µl of the 1 mM AFC conjugated substrates (50 µM final conc.) into each tube individually 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 perform the entire assay directly in a 96-well plate.
- Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.

**Note:** We recommend using a flat bottom, opaque, white or black 96-well plate for enhanced sensitivity.

**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 Colorimetric Assay Kit & Reagents
  - 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 Activity Assay & Drug Discovery Kits
  - PLTP Activity Assay & Drug Discovery Kits
  - Total Cholesterol Quantification Kit
- Molecular Biology & Reporter Assays
  - siRNA 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
- Growth Factors and Cytokines
  - Monoclonal and Polyclonal Antibodies

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

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 bacterial/ 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.