

Caspase-Family Colorimetric Substrate Set Plus

CATALOG #: K136-7-25
LOT #: _____
STORAGE CONDITIONS: Store at -20°C.
SHELF LIFE: 6 months under proper storage conditions

KIT CONTENTS:

Concentration	Description	Volume	Part Number
4 mM	Caspase-1 Substrate, Ac-YVAD-pNA	125 µl	K111-25-3
4 mM	Caspase-2 Substrate, Ac-VDVAD-pNA	125 µl	K117-25-3
4 mM	Caspase-3 Substrate, Ac-DEVD-pNA	125 µl	K106-25-3
4 mM	Caspase-5 Substrate, Ac-WEHD-pNA	125 µl	K123-25-3
4 mM	Caspase-6 Substrate, Ac-VEID-pNA	125 µl	K115-25-3
4 mM	Caspase-8 Substrate, Ac-IETD-pNA	125 µl	K113-25-3
4 mM	Caspase-9 Substrate, Ac-LEHD-pNA	125 µl	K119-25-3
N/A	Cell Lysis Buffer	100 ml	1067-100
N/A	Dilution Buffer	160 ml	K111-200-5
N/A	2X Reaction Buffer	20 ml	1068-20
1 M	DTT	0.4 ml	K111-100-4

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 10⁶ cells.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# 1067-100, -400) and incubate cells on ice for 10 minutes. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant to a fresh tube and assay protein concentration.
6. Dilute 100-300 µg protein to 50 µl Cell Lysis Buffer for each assay.
7. Add 50 µl of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
8. Add 5 µl of the 4 mM pNA conjugated substrates (200 µM final conc.) into each tube individually 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), or dilute sample to 1 ml with Dilution Buffer (Cat.# 1066-100, -500) and using regular cuvette (note: Dilution of the samples proportionally decreases the reading).
Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.

Note: 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 activity.

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

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 bacterial/ 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.