

# Caspase-5 Colorimetric Assay Kit

(Catalog #ALX-850-217, 25 tests Store kit at -20°C)

## I. Introduction:

The **Caspase-5 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspase-5 and related caspases that recognize the sequence WEHD. The assay is based on spectrophotometric detection of the chromophore *p*-nitroaniline (*p*NA) after cleavage from the labeled substrate WEHD-*p*NA. The *p*NA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400- or 405-nm. Comparison of the absorbance of *p*NA from a treated sample with an untreated control allows determination of the fold increase in Caspase-5 activity.

## II. Kit Contents:

Components	ALX-850-217-25	Part Number
	25 assays	
Cell Lysis Buffer	25 ml	ALX-K123-25-1
2X Reaction Buffer	2 ml	ALX-K123-25-2
WEHD- <i>p</i> NA (4 mM)	125 µl	ALX-K123-25-3
DTT (1 M)	100 µl	ALX-K123-25-4
Dilution Buffer	25 ml	ALX-K123-25-5

## III. Caspase-5 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 µl of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).

### B. Assay Procedure

1. Induce apoptosis by desired method. Concurrently incubate a control culture *without* treatment.
2. Pellet 2-5 x 10<sup>6</sup> cells.
3. Resuspend in 50 µl of chilled Cell Lysis Buffer and incubate on ice for 10 min.
4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and then keep on ice.
6. Assay protein concentration.
7. Dilute 100-200 µg protein to 50 µl Cell Lysis Buffer for each assay.
8. Add 50 µl 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 4 mM WEHD-*p*NA substrate (200 µM final conc.). 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 and use regular cuvette (note: Dilution of the samples proportionally decreases the reading).

You may also perform the assay in a 96-well plate.

Fold-increase in Caspase-5 activity can be determined by comparing the results of treated samples with the level of the untreated control.

**Note:** Background reading from cell lysates and buffers should be subtracted from the readings of both treated and the untreated samples before calculating fold increase in Caspase activity.

## IV. Storage and Stability:

Store kit at -20°C (Store Cell Lysis Buffer, 2X Reaction Buffer, and Dilution Buffer at 4°C after opening.). Protect WEHD-*p*NA from light. All kit components 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 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 & 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

### Growth Factors and Cytokines

- Monoclonal and Polyclonal Antibodies

**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>
<p><b>Note:</b> The most probable cause is listed under each section. Causes may overlap with other sections.</p>		