

Caspase-1/ICE Colorimetric Assay Kit

(Catalog #ALX-850-211; 25 tests Store kit at -20°C)

I. Introduction:

Activation of ICE-family proteases/caspases initiates apoptosis or other cellular processes in mammalian cells. The **Caspase-1/ICE Colorimetric Protease Assay Kits** provide a simple and convenient means for assaying the activity of caspases that recognize the sequence YVAD. The assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (*p*NA) after cleavage from the labeled substrate YVAD-*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-1 activity.

II. Kit Contents:

Components	25 assays	Part Number
Cell Lysis Buffer	25 ml	K111-25-1
2X Reaction Buffer	2 ml	K111-25-2
YVAD- <i>p</i> NA (4 mM)	125 µl	K111-25-3
DTT (1 M)	100 µl	K111-25-4
Dilution Buffer	25 ml	K111-25-5

III. Caspase-1 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 (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).
- After thawing, store Cell Lysis Buffer, 2X Reaction Buffer, and Dilution Buffer at 4°C.
- Protect YVAD-*p*NA from light.

B- Assay Procedure

1. Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture *without* treatment.
Note: Enzo's Active Recombinant Caspase-1 (Cat.# ALX-201-056) can be used as a positive control for the caspase-1 activity assays.
2. Pellet 2-5 x 10⁶ cells or use 100-200 µg cell lysates if protein concentration has been measured.
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 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 of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 4 mM YVAD-*p*NA substrate (200 µM final conc.). Incubate at 37°C for 1-2 hours.

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 entire assay directly in a 96-well plate.

Fold-increase in Caspase-1 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-1 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). All reagents are stable for at least 6 months.

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
- 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

Cell Damage & Repair

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

Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP & 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

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.		