Caspase-1/ICE Colorimetric Assay Kit

(Catalog #K111-25, -100, -200, -400; 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	K111-25 25 assays	K111-100 100 assays	K111-200 200 assays	K111-400 400 assays	Part Number
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K111-XX(X)-1
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K111-XX(X)-2
YVAD-*p*NA (4 mM)	125 µl	0.5 ml	2 x 0.5 ml	2 x 1 ml	K111-XX(X)-3
DTT (1 M)	100 µl	0.4 ml	0.4 ml	0.4 ml	K111-XX(X)-4
Dilution Buffer	25 ml	100 ml	200 ml	400 ml	K111-XX(X)-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: BioVision's Active Recombinant Caspase-1 (Cat.# 1081-25, -100) can be used as a positive control for the caspase-1 activity assays.
2. Pellet 2-5 x 10^6 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.

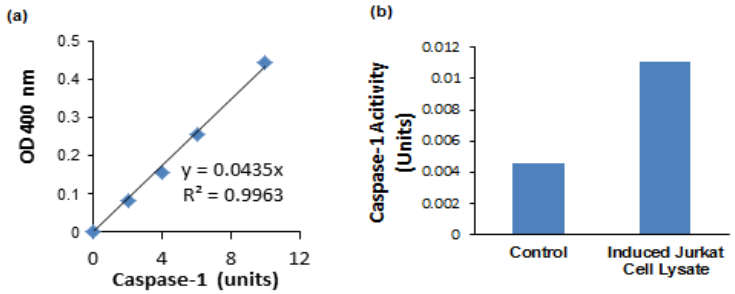

Figure: (a) Active Recombinant Caspase-1 (Cat # 1081) Standard Curve. (b) Caspase-1 activity in 1 µg uninduced and induced Jurkat cell lysate. Assay was performed following the kit protocol.

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

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	• Cells did not lyse completely	• Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet
	• Experiment was not performed at optimal time after apoptosis induction	• Perform a time-course induction experiment for apoptosis
	• Plate read at incorrect wavelength	• Check the wavelength listed in the datasheet and the filter settings of the instrument
	• Old DTT used	• Always use freshly thawed DTT in the cell lysis buffer
High Background	• Increased amount of cell lysate used	• Refer to datasheet and use the suggested cell number to prepare lysates
	• Increased amounts of components added due to incorrect pipetting	• Use calibrated pipettes
	• Incubation of cell samples for extended periods	• Refer to datasheet and incubate for exact times
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the individual components appropriately
	• Contaminated cells	• Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	• Cells did not initiate apoptosis	• Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	• Very few cells used for analysis	• Refer to datasheet for appropriate cell number
	• Use of samples stored for a long time	• Use fresh samples or aliquot and store and use within one month for the assay
	• Incorrect setting of the equipment used to read samples	• Refer to datasheet and use the recommended filter setting
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	• Uneven number of cells seeded in the wells	• Seed only equal number of healthy cells (correct passage number)
	• Samples prepared in a different buffer	• Use the cell lysis buffer provided in the kit
	• Adherent cells dislodged and lost at the time of experiment	• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope
	• Samples used after multiple freeze-thaw cycles	• Aliquot and freeze samples, if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot as needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Cell samples contain interfering substances	• Troubleshoot if it interferes with the kit (run proper controls)
General issues	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Incorrect incubation times or temperatures	• Refer to datasheet & verify the correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
	• Air bubbles formed in the well/tube	• Pipette gently against the wall of the well/tubes
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
	• Use of a different 96-well plate	• Fluorescence: Black plates; Absorbance: Clear plates

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