Induced Jurkat



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	K111-100	K111-200	K111-400	Part Number
	25 assays	100 assays	200 assays	400 assays	
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-pNA (4 mM)	125 µl	0.5 ml	2 x 0.5 ml	2 x 1 ml	K111-XX(X)-3
DTT (1 M)	100 μΙ	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-pNA from light.

B. Assay Procedure

 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.
- Add 50 μl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 μl of the 4 mM YVAD-pNA substrate (200 μM final conc.). Incubate at 37 C for 1-2 hours.
- 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.

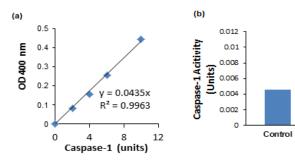


Figure: (a) Active Recombinant Caspase-1 (Cat # 1081) Standard Curve. (b) Caspase-1 activity in 1 μ g uninduced and induced Jurkat cell lysate. Assay was performed following the kit protocol.

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	Perform a time-course induction experiment for apoptosis		
	induction • 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	Use calibrated pipettes		
	pipettingIncubation 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		

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Page 2