Caspase-3/CPP32 Colorimetric Assay Kit

(Catalog #K106-25, -100, -200, -400; Store kit at –20'C)

I. Introduction:

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **Caspase-3/CPP32 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence DEVD. The assay is based on spectrophotometric detection of the chromophore *p*-nitroaniline (*p*NA) after cleavage from the labeled substrate DEVD-*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 an apoptotic sample with an uninduced control allows determination of the fold increase in CPP32 activity.

II. Kit Contents:

Components	K106-25	K106-100	K106-200	K106-400	Part Number
	25 assays	100 assays	200 assays	400 assays	
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml	K106-XX(X)-1
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml	K106-XX(X)-2
DEVD-*p*NA (4 mM)	125 µl	0.5 ml	2 x 0.5 ml	2 x 1 ml	K106-XX(X)-3
DTT (1 M)	100 µl	0.4 ml	0.4 ml	0.4 ml	K106-XX(X)-4
Dilution Buffer	25 ml	100 ml	200 ml	400 ml	K106-XX(X)-5

III. Caspase-3 Assay Protocol:

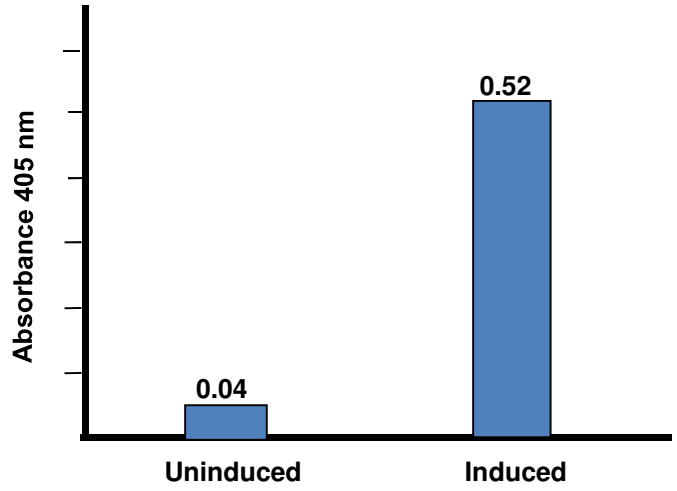
A. General Considerations

- 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).
- Protect DEVD-*p*NA from light.

B. 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 10^6 cells.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice for immediate assay or aliquot and store at –80˚C for future use.
6. Assay protein concentration.
7. Dilute 50-200 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.
9. Add 5 µl of the 4 mM DEVD-*p*NA substrate (200 µM final conc.) and incubate at 37˚C for 1-2 hour.
10. 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 using regular cuvette (note: Dilution of the samples proportionally decreases the reading). Fold-increase in CPP32 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 CPP32 activity



Induction of Caspase-3 Activity by Anti-Fas Antibody in Jurkat –T Cells Using Caspase-3 Colorimteric Assay Kit K106-25

IV. Storage and Stability:

Store kit at –20˚C (Store Lysis Buffer, Reaction Buffer, and Dilution Buffer at 4˚C after opening). All reagents are stable for at least 6 months under proper storage conditions.

VI. 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

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

GENERAL TROUBLESHOOTING GUIDE FOR CASPASE COLORIMETRIC AND FLUORIMETRIC 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 / assay not done at optimal timepoint after induction of 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 cells 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 uniformly suspended 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	• Trouble-shoot 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	• Trouble shoot 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 or spin down the plate
	• 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.		