

# 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*-nitroanilide (*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
	25 assays	100 assays	200 assays	400 assays
Cell Lysis buffer	25 ml	100 ml	100 ml	100 ml
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml
DEVD- <i>p</i> NA (4 mM)	125 µl	0.5 ml	2 x 0.5 ml	2 x 1 ml
DTT (1 M)	100 µl	0.4 ml	0.4 ml	0.4 ml
Dilution Buffer	25 ml	100 ml	200 ml	400 ml

## 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<sup>6</sup> 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 cuvet (Sigma), or dilute sample to 1 ml with Dilution Buffer and using regular cuvet (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 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.

## 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

### Cell Damage & Repair

- HDAC & HAT Fluorometric & Colorimetric Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione, GST, & Nitric Oxide Fluorometric & Colorimetric Assay Kits

### Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

### Adiponectin & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin

