

Caspase-3/ CPP32 Fluorometric Assay Kit

(Catalog #K105-25, -100, -200, -400; Store kit at -20°C)

I. Introduction:

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **CPP32/Caspase-3 Fluorometric Protease Assay Kit** provides a simple and convenient means for assaying the DEVD-dependent caspase activity. The assay is based on detection of cleavage of substrate DEVD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). DEVD-AFC emits blue light ($\lambda_{max} = 400$ nm); upon cleavage of the substrate by CPP32 or related caspases, free AFC emits a yellow-green fluorescence ($\lambda_{max} = 505$ nm), which can be quantified using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase-3/ CPP32 activity.

II. Kit Contents:

Components	K105-25	K105-100	K105-200	K105-400
	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-AFC (1 mM)	125 μ l	500 μ l	2 x 0.5 ml	2 x 1 ml
DTT (1 M)	100 μ l	400 μ l	400 μ l	400 μ l

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 μ l of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).
- After thawing, store the Cell Lysis Buffer and 2X Reaction Buffer at 4°C.
- Protect DEVD-AFC 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 $1-5 \times 10^6$ cells or use 20-200 μ g cell lysates (depending on the apoptosis level).

Notes: 1) For tissue samples, tissue can be homogenized in Lysis Buffer (for 1X volume of tissue, add 3X volume of lysis buffer) to generate tissue lysate, then follow the kit procedure.

2) Tissue and cell lysates can be kept frozen at -80°C for up to 2 months without significant loss of activity.

3. Resuspend cells in 50 μ l of chilled Cell Lysis Buffer.
4. Incubate cells on ice for 10 minutes.
5. Add 50 μ l of 2X Reaction Buffer (containing 10 mM DTT) to each sample.
6. Add 5 μ l of the 1 mM DEVD-AFC substrate (50 μ M final concentration) and incubate at 37°C for 1-2 hour.

7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. 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.

IV. Storage and Stability:

Store kit at -20°C (Store Lysis Buffer and 2X Reaction Buffer at 4°C after opening). All reagents are stable for 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
- Apoptosis siRNA Vectors

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

Signal Transduction

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

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- PLTP Activity Assay Kit
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays

- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits