Caspase 3 Assay Kit, Colorimetric

***Storage*：**

Components	Storage	Valid
Cell Lysis Buffer	RT	1 year
Ac-DEVD-*p*NA	-20°protect form light	1 year
Assay Buffer	RT	1 year
DTT	-20°	1 year

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description

Activation of caspase family proteases/caspases initiates apoptosis in mammalian cells. The Caspase-3 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 (pNA) after cleavage from the labeled substrate DEVD-pNA. The pNA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400- or 405-nm. Comparison of the absorbance of pNA from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase3 activity.

Protocol：

A. General Considerations

Aliquot enough Cell Lysis Buffer and Assay Buffer for the number of assays to be performed. Add DTT to Buffer immediately before use (Final concentration: add 10 μl of 1.0 M DTT stock per 1 ml of Cell Lysis Buffer and Assay Buffer). Protect DEVD-pNA 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 2-5 x 10^6 cells.
3. Resuspend cells in 100 μl of chilled Cell Lysis Buffer and incubate cells on ice for 15 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 20-50 μg protein to 10 μl Cell Lysis Buffer for each assay.
8. Add 90 μl of Assay Buffer (containing 1/100 DTT) to each sample.
9. Add 10 μl of the DEVD-pNA substrate and incubate at 37° C for 1-2 hour.
10. Read samples at 400- or 405-nm in a microtiter plate reader

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