Caspase 8 Assay Kit, Colorimetric

***Storage*：**

Components	Storage	Valid
Cell Lysis Buffer	RT	1 year
Ac-IETD-*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 ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **Caspase-8 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence IETD. The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate IETD-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 caspase 8 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 IETD-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 IETD-pNA substrate and incubate at 37° C for 1-2 hour. Protect IETD-pNA from light
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 caspase 8 activity