Caspase-8 Colorimetric Assay Kit

I. DESCRIPTION

The Caspase-8 Colorimetric Protease Assay Kit provides a simple and convenient way for assaying the activity of caspase-8. Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The assay for caspase-8 is based on spectrophotometric detection of the chromophore *p*nitroanilide (*p*NA) after cleavage from the labeled substrate IETD-*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 un-induced control, it can determine the fold increase of Caspase-8 activity.

II. KEY FEACTURE

- **Easy to Perform:** simple procedures and ready-to-use to perform.
- **Reproducible results:** The kit produces highly reproducible results.
- **Less time to use**

III. KIT CONTENTS

The Caspase-8 Colorimetric Assay Kit is available for detection using the labeled substrate IETD–*p*NA.

Components	**Cat:** EBA3150 （50 assays）	**Cat:** EBA31100 （100 assays）
Lysis Buffer	10.0 mL	15.0 mL
2×Reaction Buffer	2.5 mL	5.0 mL
Caspase-8 Substrate	250μL	500μL
DTT	100 μL	150 μL

IV. STORAGE

Store the kit at -20℃, while store the Lysis Buffer and 2×Reaction Buffer at 4℃ after opened. All reagents will remain stable for one year.

V. CASPASE-8 COLORIMETRIC ASSAY KIT PROTOCOL

A. General Considerations

- Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT into the 2X Reaction Buffer immediately to 10 mM final concentration before use (add 10 μl of 1.0 M DTT stock in per 1ml 2X Reaction Buffer).
- Store the Lysis Buffer at 4℃ after thawing.
- Do not expose Caspase-8 Substrate from light.

B. Assay Procedure

1. Induce cells apoptosis with required way and set negative control without induction at the same time.

2. Collect cells and wash cells twice with PBS by centrifugation in 2000 rpm for 5 min.
3. Collect 3～5×10^6 cells, try to remove PBS
4. Add 50μl cold prepared Lysis Buffer into collected cells and mix uniformity. (Note: Before using Lysis Buffer, add 0.5 μl DTT into 50 μl Lysis Buffer)
5. Incubate cells on ice for 20～60 min, vortex vibration 3-4 times,10 s per time, or freeze thawing 2-3 times.
6. Centrifugation in 10,000 rpm at 4 ℃ for 1 min.
7. Suck supernatant and transfer to a new tube, put it on ice
8. Get a small quantity of supernatant to assay the protein concentration by Bradford or BCA method.
9. Take 50 μl supernatant containing 100～200 μg protein. If there is no enough volume, Lysis Buffer can be compensated.
10. Add 50 μl of 2X Reaction Buffer to each sample. (Note: add 0.5 μl DTT into 50 μl 2×Reaction Buffer)
11. Add 5 μl Caspase-8 Substrate (200 μM final conc.) and incubate at 37°C for 1～2 hour, away from light.
12. Determine extinction value of samples by a spectrophotometer (using a 100 μl micro quartz cuvet) or a microtiter plate reader at 400 nm or 405nm. Get the result of the induced group's Caspase-8 activity by computing $OD_{inducer}/OD_{negtive\ control}$.

Note: Background of cell lysates buffers from the readings of induced samples and negative control should be subtracted before calculating fold increase in Caspase-8 activity.