



Caspase 9 Assay Kit, Colorimetric

Storage:

Components	Storage	Valid
Cell Lysis Buffer	RT	1 year
Ac-LEHD-pNA	-20°protect from 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-9 Colorimetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the sequence LEHD. The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate LEHD-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-9 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 μ l of 1.0 M DTT stock per 1 ml of Cell Lysis Buffer and Assay Buffer). Protect LEHD-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 10⁶ cells.
3. Resuspend cells in 100 μ l 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 μ g protein to 10 μ l Cell Lysis Buffer for each assay.
8. Add 90 μ l of Assay Buffer (containing 1/100 DTT) to each sample.
9. Add 10 μ l of the LEHD-pNA substrate and incubate at 37° C for 1-2 hour. Protect LEHD-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 9 activity