

NutriCulture Colorimetric Caspase-8 Activity Assay Kit

100 tests

Cat No: C8A100

Shipping : Ship with blue/dry ice.

Storage : Store at -20°C. It is recommended to aliquot the NutriCulture IETD-pNA into smaller quantities and store in the dark. Avoid repeated freezing and thawing.

General Information

Activation of Caspase-8 initiates apoptosis in mammalian cells. The NutriCulture Colorimetric Caspase-8 Activity Assay Kit provides a simple and convenient means for assaying the activity of Caspase-8 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

General Considerations

It is recommended that the number of cells for one sample should not be less than 1×10^6 , make sure that the protein concentration is 1~4 mg/mL. Otherwise, it will affect the accuracy of the experiment, resulting in low protein concentration, low activity of Caspase-8 in the reaction system, and low OD value.

Aliquot enough NutriCulture 2x Reaction Buffer for the number of assays to be performed. Add DTT to the NutriCulture 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).

Aliquot enough NutriCulture Cell Lysis Buffer for the number of assays to be performed. Add DTT to the NutriCulture Lysis Buffer immediately before use (5 mM final concentration: add 5 μ l of 1.0 M DTT stock per 1 ml of NutriCulture Cell Lysis Buffer). Add PMSF (not provided) to the NutriCulture Lysis Buffer immediately before use (1 mM final concentration: add 10 μ l of 100 mM PMSF stock per 1 ml of NutriCulture Cell Lysis Buffer).

Assay Procedure

1. Grow cells at a 70% - 80% density.
2. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
Note: Caspase 8 activation can be induced by incubating cells with anti-CD95 (Fas) antibody as positive control.
3. Gently scrape cells in culture media and spin down cell pellet at 200 x g for 10 minutes.
4. Wash cells in cold PBS.
5. Count and pellet $1 - 5 \times 10^6$ cells.
6. Resuspend cells in 50 μ L of ice cold NutriCulture Cell Lysis Buffer.
7. Homogenize cells quickly by pipetting up and down a few times and incubate sample on ice for 10 – 30 minutes.
8. Centrifuge for 10 minutes at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

For further information;
ecotechbiotech.com
info@ecotechbiotech.com

9. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice.

10. Measure protein concentration and adjust 50-200 µg protein to 45 µl *NutriCulture* Cell Lysis Buffer for each sample.

11. Add 50 µl of *NutriCulture* 2x Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the *NutriCulture* IETD-pNA substrate. Mix well by pipetting up and down. Incubate at 37°C for 1-2 hours protected from light.

Note: Assay all controls and samples at least in duplicates.

12. Read samples at 400- or 405-nm in a microplate reader.

Note: Background reading (blank) 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.