Tribioscience

Caspase-1/ICE Fluorometric Assay (Catalog: TBS2045, 100 Assays, Store at -20 ℃)

DESCRIPTION

Caspase-1 (IL-1β Converting Enzyme, ICE) is the prototypical member of the ICE family of cysteine proteases, also known as caspases (**c**ysteinyl **a**spartate-**sp**ecific prote**ases**). Caspase-1 was first identified as a novel protease that generates the proinflammatory cytokine, interleukin-1β by cleaving pro-interleukin-1β. It plays an important role in inflammatory diseases and cancer.

Tribo™ Caspase-1 Activity Fluorometric Assay Kit provides a simple and convenient method for assaying the activity of caspases that recognize the sequence YVAD. The assay is based on the detection of the free AFC fluorescence after cleavage from the labeled substrate YVAD-AFC. The free AFC can be quantified using a fluorometer or a fluorescence microtiter plate reader at Ex400/Em505nm. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows the determination of the fold increase in caspase-1 activity.

KIT CONTENTS for 100 assays

Component	Volume
Cell Lysis Buffer	12 mL
Assay Buffer	6 mL
Caspase-1 Substrate	600 µL
DTT	200 µL

Store at -20°C. Shelf life is 1 year.

ASSAY PROTOCOL

1. Treatment cells by desired method including no induction control. We recommend adding caspase-1 enzyme as a positive control: 1-2U positive control /50 µL/well.
2. Count cells and pellet 2-5x10^6 cells in 1.5 mL tubes. 3. Resuspend cells in 50 µL of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
3. Centrifuge for 1 min (10,000 x g).
4. 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.
5. Optional: Measure protein concentration (Protein assay kit, TBS2005).
6. Adjust sample protein concentration to 50-200 µg protein in 50 µL/well with Lysis Buffer.
7. Immediately before use, prepare enough working reagent by per assay add 50 µL Assay buffer, and 1 µL DTT.
8. Transfer 50µL working reagent into each well.
9. Add 5 µL Caspase-1 Substrate into each well, fully mix.
10. Transfer 50µL sample protein, positive, blank into the indicated well in duplicate manner.
11. Incubate at 37°C for 1-2hr, protecting from light.
12. Read plate at Ex400/Em505nm in a fluorescence plate reader.
13. Fold-increase in Caspase-1 activity can be determined by comparing these results with the level of uninduced control.
14. 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-1 activity.

RELATED PRODUCTS

Resazurin Cell Viability Assay (TBS2001)
LDH Cytotoxicity Colorimetric Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay (TBS2020)
Protein Assay (TBS2005)
Caspase-1 Colorimetric Assay (TBS2040)
Caspase-3 Colorimetric Assay (TBS2030)
Caspase-3 Fluorometric Assay (TBS2035
CCK-8 Cell Viability Assay (TBS2022)
GOT Activity Assay (TBS2013)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Inhibitor Screening Assay (TBS2097)
G6PDH Activity Colorimetric Assay (TBS2102)

This product is for research use only.

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