For research use only

Caspase-5 Fluorometric Substrate, WEHD-AFC

CATALOG #: 1101-200 200 assays (2 x 0.5 ml)
1101-1000 1000 assays (5 x 1 ml)

LOT #: _____

STORAGE: Store at –20°C, protected from light.

SHELF LIFE: 1 year under proper storage conditions

MOL. WEIGHT: 952.0

SEQUENCE: Ac-Trp-Glu-His-Asp-AFC
(AFC, 7-amino-4-trifluoromethyl coumarin)

PURITY: >99% by HPLC analysis.

DESCRIPTION:

Ready-to-use fluorometric substrate for caspase-1,-4,-5 and related caspases that recognize the amino acid sequence WEHD. Caspase-5 and related caspase activity can be quantified by fluorescent detection of free AFC after cleavage from the peptide substrate WEHD-AFC at Ex. = 400 nm and Em. = 505 nm, using a fluorometer or multi-well fluorescence plate reader. Alternatively, a shift in fluorescence from blue to green upon cleavage can be visualized using a hand-held long-UV lamp. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large amount of caspase assays. Cell Lysis Buffer (Cat. #1067-100, -400) and 2X Reaction Buffer (Cat. #1068-20, -80) for caspase assays are also available separately.

ASSAY PROTOCOL:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. Count cells and pellet 1-5 x 10^6 cells or use 50-200 µg cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# 1067-100).
4. Incubate cells on ice for 10 minutes.
5. Add 50 µl of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
6. Add 5 µl of the 1 mM WEHD-AFC (50 µM final conc.) into each tube individually and incubate at 37°C for 1-2 hour.
7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may perform the entire assay directly in a 96-well plate.

   Fold-increase in caspase-5 activity can be determined by comparing these results with the level of the uninduced control.

VI. Related Products:

1. KinaseSTAR™ JNK & Akt Activity Assay Kit
2. Histone Deacetylase Fluorometric & Colorimetric Assay Kits
3. Calpain Activity Assay Kit
4. Apoptotic Cell Isolation Kit
5. MitoCapture™ Mitochondrial Apoptosis Detection Kit
6. Mitochondria/Cytosol Fractionation Kit
7. Nuclear/Cytosol Fractionation Kit
8. Cytochrome c Releasing Apoptosis Assay Kit
9. ApoSENSOR™ ATP Determination Kit & ADP/ATP Ratio Assay Kit
10. ApoGSH™ Glutathione Detection Kit
11. Nitric Oxide Fluorometric & Colorimetric Detection Kits
12. Annexin V-FITC, -Cy3, -Cy5, -EGFP, -PE, -Biotin Kits and Bulk Reagents
13. CaspSCREEN™ Flow Cytometric Caspase Assay Kit
14. CaspGLOW™ Active Caspase Staining Kits
15. CaspSELECT™ Caspase-3 & -7 Immunoassay Kits
16. Caspase-1,-2,-3,-5,-6,-8,-9,-10 Activity Assay Kits
17. Recombinant Human, Mouse, Rat Active Caspases & Procaspases
18. Ready-to-use Caspase Inhibitors & Sets
19. Ready-to-use Caspase Substrates and Sets
20. Quick Apoptotic DNA Ladder Detection Kit
21. Ready-to-use Apoptosis Inducers and Set
22. Quick Cell Proliferation Assay Kit
23. LDH-Cytotoxicity Assay Kit
24. Live-Dead Cell Staining Kit
25. Anti-Caspase-1,-2,-3,-4,-5,-6,-7,-8,-9,-10,-11,-12,-13,-14 & Active Caspases
26. Many Other Apoptosis & Cell Signaling Related Antibodies
27. Growth Factors, Cytokines & Chemokines (many)
28. Link-FAST™ 5 Minutes DNA Ligation Kit
29. Gel-FAST™ 20 Minutes Gel Staining/Destaining Kit