

## Caspase-9 Fluorometric Substrate, LEHD-AFC

**CATALOG #:** 1075-200 200 assays (2 x 0.5 ml)  
1075-1000 1000 assays (5 x 1 ml)

**LOT #:** \_\_\_\_\_

**STORAGE:** Store at -20°C, protected from light. Stable for 6 months

**MOL. WEIGHT:** 765.0

**SEQUENCE:** Ac-Leu-Glu-His-Asp-AFC

**PURITY:** >98% by HPLC analysis.

### DESCRIPTION:

Ready-to-use fluorometric substrate for caspase-9/Mch-6 and related caspases that recognize the amino acid sequence LEHD. Caspase activity can be quantified by fluorescent detection of free AFC after cleavage from the peptide substrate LEHD-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) and DTT (Cat.# 1201-1) 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  $1-5 \times 10^6$  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 LEHD-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 LEHD-dependent 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 & Procaspsases
18. Ready-to-use Caspase Inhibitors & Sets
19. Ready-to-use Caspase Substrates and Sets
20. Quick Apoptotic DNA Ladder Detection Kit
21. TUNEL-based DNA Fragmentation Assay Kits
22. Ready-to-use Apoptosis Inducers and Set
23. Quick Cell Proliferation Assay Kit
24. LDH-Cytotoxicity Assay Kit
25. Live-Dead Cell Staining Kit
26. Anti-Caspase-1,-2,-3,-4,-5,-6,-7,-8,-9,-10,-11,-12,-13,-14 & Active Caspases
27. Anti-Apaf-1, Bcl-2, Bcl-x, Bax, Bad, Bid, Bim/Bod, Mcl-1, Cytochrome c
28. Anti-DFF40, DFF45, FADD, p53, IRAK, Wee1, CD40, DcR2, cFLIP, CTGF
29. Many Other Apoptosis & Cell Signaling Related Antibodies
30. Anti-GFP, His, GST, and Other Tag Antibodies
31. Growth Factors, Cytokines & Chemokines (many)
32. Link-FAST™ 5 Minutes DNA Ligation Kit
33. Gel-FAST™ 20 Minutes Gel Staining/Destaining Kit
34. Protease Inhibitor Cocktail