

## 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 x 10<sup>6</sup> 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.

### RELATED PRODUCTS:

#### Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA Vectors

#### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

#### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

#### Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

#### Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

#### Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

#### Molecular Biology & Reporter Assays

- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit
- β-Galactosidase Staining Kit & Luciferase Reporter Assay Kit

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies