

## FLICE/Caspase-8 Fluorometric Substrate, IETD-AFC

**CATALOG NO:** 1062-200 200 assays (1 x 1.0 ml)  
1062-1000 1000 assays (5 x 1.0 ml)

**STORAGE:** Store at  $-20^{\circ}$  C, protected from light.

**SHELF LIFE:** 1 year under proper storage conditions

**CONCENTRATION:** 2 mM in DMSO

**MOL. WEIGHT:** 729.0

**SEQUENCE:** Ac-Ile-Glu-Thr-Asp-AFC  
(AFC, 7-amino-4-trifluoromethyl coumarin)

**PURITY:** >95% by HPLC analysis.

### DESCRIPTION:

Ready-to-use fluorometric substrate for FLICE/caspase-8 and related caspases that recognize the amino acid sequence IETD. The sequence IETD is based on caspase-8 cleavage site in CPP32/caspase-3 proenzyme. FLICE and related caspase activity can be quantified by fluorescent detection of free AFC after cleavage from the peptide substrate IETD-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.

### 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  $\mu$ g cell lysates if protein concentration has been measured.
3. Resuspend cells in 50  $\mu$ l of chilled Cell Lysis Buffer (Cat.# 1067-100).
4. Incubate cells on ice for 10 minutes.
5. Add 50  $\mu$ l of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
6. Add 5  $\mu$ l of the 2 mM IETD-AFC (100  $\mu$ M final conc.) into each tube individually and incubate at  $37^{\circ}$  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.
8. Fold-increase in IETD-dependent caspase-8 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
- $\beta$ -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies