

Caspase-1 Fluorometric Substrate, YVAD-AFC

CATALOG NO:	1103-200 200 assays (1 x 1 ml) 1103-1000 1000 assays (5 x 1 ml)
STORAGE:	Store at -20° C, protected from light.
SHELF LIFE:	6 months under proper storage conditions
MOL. WEIGHT:	719.7
SEQUENCE:	Ac-Tyr-Val-Ala-Asp-AFC (AFC, 7-amino-4-trifluoromethyl coumarin)
PURITY:	>98% by HPLC analysis.

DESCRIPTION:

Ready-to-use fluorometric substrate for caspase-1/ICE and related caspases that recognize the amino acid sequence YVAD. Caspase-1 and related caspase activity can be quantified by fluorescent detection of free AFC after cleaved from the peptide substrate YVAD-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 volume caspase assays. Cell Lysis Buffer (Cat. #1067-100, -400) and 2X Reaction Buffer (Cat. #1068-20, -80), and DTT used for caspase assays are also available separately.

ASSAY PROTOCOL:

1. Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture *without* treatment.
Note: Active recombinant human caspase-1 is available to use as a positive control (BioVision, Cat.# 1081-25, -100).
2. Pellet 2-5 x 10⁶ cells or use 100-300 µg cell lysates if protein concentration has been measured.
4. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# 1067-100).
5. Incubate cells on ice for 10 minutes.
6. Add 50 µl of 2X Reaction Buffer (Cat.# 1068-20,-80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
7. Add 5 µl of the 1 mM YVAD-AFC substrate (50 µM final concentration) and incubate at 37° C for 1-2 hour.
8. Read samples in a fluorometer equipped with a 400-nm excitation and 505-nm emission filters. For a plate-reading set-up, transfer the samples to a 96-well plate. You may also perform the entire assay directly in a 96-well plate. Fold-increase in YVAD-dependent caspase activity can be determined by comparing these results with the level of the untreated 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

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