Fluorogenic and Chromogenic Caspase Substrates

Biological Applications

Caspases are essential in cells for apoptosis, or programmed cell death, in development and most other stages of adult life, and have been termed "executioner" proteins for their roles in the cell. Some caspases are also required in the immune system for the maturation of lymphocytes. Failure of apoptosis is one of the main contributions to tumour development and autoimmune diseases. Caspases are also involved in ischemia and Alzheimer's disease. There are two types of apoptotic caspases: initiator and effector caspases. Initiator caspases (e.g., CASP2, CASP8, CASP9, and CASP10) cleave inactive pro-forms of effector caspases, thereby activating them. Effector caspases (e.g., CASP3, CASP6, CASP7) in turn cleave other protein substrates within the cell, to trigger the apoptotic process. The initiation of this cascade reaction is regulated by caspase inhibitors.

AAT Bioquest offers both color and a group of blue, green, and red fluorescent substrates for monitoring caspase activities. The AMC-, AFC-, R110- and ProRed™-derived protease substrates are colorless and non-fluorescent. Cleavage of blocking protease-cleavable peptide residue by caspases generates the strongly blue, green, or red fluorescent respectively that can be monitored by fluorescence instruments. Our proprietary ProRed™-derived caspase substrates are the most sensitive red indicators for the fluorimetric detection of various caspase activities. In general, R110 and ProRed™ substrates are much more sensitive than the AMC-, AFC- or 4-nitroaniline-based substrates.

Storage Conditions

Store at < -15 °C and desiccated. Avoid exposure to light. Expiration date is one year upon receipt.

Physical and Spectral Properties

Cat#	Indicators	Function (for Caspases)	Unit	MW	Solvent	Ex (nm)	Em (nm)
13401	Ac-DEVD-AFC	Caspase 3/7	5 mg	729.61	DMSO	380	500
13402	Ac-DEVD-AMC	Caspase 3/7	5 mg	675.64	DMSO	351	430
13403	Ac-DEVD-CHO	Caspase 3/7 inhibitor	1 mg	502.47	DMSO	N/A	N/A
13405	Ac-DEVD-pNA	Caspase 3/7	5 mg	638.58	DMSO	408	N/A
13406	FITC-C6-DEVD-FMK	Caspase 3/7 (cell permeable)	1 mg	994.99	DMSO	492	516
13408	FITC-C6-DEVD-FMK	Caspase 3/7 (cell permeable)	100 µg	994.99	DMSO	492	516
13407	FITC-C6-LEHD-FMK	Caspase 9 (cell permeable)	1 mg	1031.07	DMSO	492	516
13409	FITC-C6-LEHD-FMK	Caspase 9 (cell permeable)	100 µg	1031.07	DMSO	492	516
13410	Ac-IETD-AFC	Caspase 8	5 mg	729.65	DMSO	380	500
13411	Ac-IETD-AMC	Caspase 8	5 mg	675.68	DMSO	351	430
13412	Ac-IETD-CHO	Caspase 8 inhibitor	5 mg	502.52	DMSO	N/A	N/A
13413	Z-IETD-pNA	Caspase 8	5 mg	730.72	DMSO	408	N/A
13420	Z-DEVD-AFC	Caspase 3/7	5 mg	821.71	DMSO	380	500
13421	Z-DEVD-AMC	Caspase 3/7	5 mg	767.74	DMSO	351	430
13422	Z-DEVD-pNA	Caspase 3/7	5 mg	730.68	DMSO	408	N/A
13425	Z-IETD-AFC	Caspase 8	5 mg	821.75	DMSO	380	500
13426	Ac-LEHD-AMC	Caspase 9	5 mg	711.72	DMSO	351	430
13427	(Ac-LEHD)2-R110	Caspase 9	1 mg	1403.41	DMSO	498	520
13430	(Z-DEVD)2-R110	Caspase 3/7	1 mg	1515.44	DMSO	498	520
13431	(Ac-IETD)2-R110	Caspase 8	1 mg	1331.34	DMSO	498	520
13433	Z-DEVD-ProRed™ 620	Caspase 3/7	1 mg	1565.50	DMSO	534	619
13434	Z-IETD-ProRed™ 620	Caspase 8	1 mg	1565.59	DMSO	534	619
13435	Z-LEHD-ProRed™ 620	Caspase 9	1 mg	1453.47	DMSO	534	619
13470	FAM-VAD-FMK	1, 2, 3, 6, 7, 8, 9, 10 (cell permeable)	25 tests	691.66	DMSO	492	518
13471	TF4-VAD-FMK	1, 2, 3, 6, 7, 8, 9, 10 (cell permeable)	25 tests	849.94	DMSO	588	610
13472	SRB-VAD-FMK [Sulforhodamine B-VAD-FMK]	1, 2, 3, 6, 7, 8, 9, 10 (cell permeable)	25 tests	874.01	DMSO	556	575
13475	mFluor™ 450-VAD-FMK	1, 2, 3, 6, 7, 8, 9, 10 (cell permeable)	25 tests	555.94	DMSO	403	454
13476	mFluor™ 510-VAD-FMK	1, 2, 3, 6, 7, 8, 9, 10 (cell permeable)	25 tests	708.19	DMSO	414	508

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Sample Protocol

Following protocol only provides a guideline, and should be modified according to your specific needs.

1. **General Solution Caspase Assays Using AMC, AFC, pNA, R110 and ProRed Substrates**
   1.1. Prepare a 10 mM stock solution in DMSO.
   1.2. Prepare a 2X caspase substrate (50 μM) assay solution as the following:
   50 μL substrate stock solution
   100 μL DTT (1M)
   400 μL EDTA (100 mM)
   10 mL Tris Buffer (20 mM), pH =7.4
   1.3. Mix equal volume of the caspase standards or samples with 2X caspase substrate assay solution (from Step 1.2), and incubate the solutions at room temperature for at least 1 hour.
   1.4. Monitor the fluorescence using a fluorescence microplate reader, or absorbance using an absorbance microplate reader.

2. **Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes**
   2.1. Prepare a 2-5 mM stock solution in DMSO.
   2.2. Treat cells as desired.
   2.3. Prepare a 2X permeable caspase substrate (20 μM) assay solution by diluting the DMSO stock solution (from Step 2.1) in Hanks with 20 mM Hepes buffer (HHBS).
   2.4. Mix equal volume of the treated cells with 2X caspase substrate assay solution (from Step 2.3), and incubate the cells in a 37°C, 5% CO_2 incubator for at least1 hour.
   2.5. Wash the cells with HHBS for at least once.
   2.6. Monitor the fluorescence intensity by a flow cytometer, a fluorescence microscope or a fluorescence microplate reader.

3. **Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes (For #13470-13476 only)**
   3.1. Prepare a 250X stock solution by adding 50 μL DMSO into the vial.
   3.2. Treat cells as desired.
   3.3. Add 250 X DMSO stock solution (from Step 3.1) into the cell solution at a 1:250 ratio (such as 2 uL to 500 uL cells), and incubate the cells in a 37°C, 5% CO2 incubator for 1 hour.
   3.4. Wash the cells with HHBS for at least once.
   3.5. Monitor the fluorescence intensity by flow cytometer, fluorescence microscopy or fluorescent microplate reader.

References

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