

Fluorimetric and Colorimetric Caspase Substrates

Biological Applications

Caspases play important roles in apoptosis and cell signaling. They 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 fluorescent 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.

Spectral Properties

Cat#	Indicators	Function	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
13407	FITC-C6-LEHD-FMK	Caspase 9 (cell permeable)	1 mg	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

Sample Protocol

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

1. For General Caspase Assays

- 1.1. Prepare a 10 mM stock solution in DMSO.
- 1.2. Prepare a 2X caspase substrate (50 μ M) assay solution as the following:
50 μ L substrate stock solution
100 μ L DTT (1M)
400 μ L 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 use fluorescent microplate readers, or absorbance using colorimetric microplate readers.

2. For Cell Permeable Caspase Substrate Assays

- 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 μ M) 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 1.2), and incubate the cells in a 37°C, 5% CO₂ incubator for at least 1 hour.
- 2.5. Wash the cells with HHBS for at least once.
- 2.6. Monitor the fluorescence intensity by flow cytometer, fluorescence microscopy or fluorescent microplate reader.

References

1. Imre G, Heering J, Takeda AN, Husmann M, Thiede B, Zu Heringdorf DM, Green DR, van der Goot FG, Sinha B, Dotsch V, Rajalingam K. (2012) Caspase-2 is an initiator caspase responsible for pore-forming toxin-mediated apoptosis. *EMBO J*.
2. Zhang F, Lau SS, Monks TJ. (2012) A Dual Role for Poly(ADP-ribose) Polymerase-1 (PARP-1) During Caspase-dependent Apoptosis. *Toxicol Sci*.
3. Xiao XL, Peng J, Su Q, Xiang SL, Tang GH, Huang YS, Zhou XT. (2006) [Diallyl Trisulfide Induces Apoptosis of Human Gastric Cancer Cell Line MGC803 Through Caspase-3 Pathway.]. *Ai Zheng*, 25, 1247.
4. Sakaue M, Motoyama Y, Yamamoto K, Shiba T, Teshima T, Chiba K. (2006) Quantitative measurement of caspase-3 activity in a living starfish egg. *Biochem Biophys Res Commun*, 350, 878.
5. Kume T, Taguchi R, Katsuki H, Akao M, Sugimoto H, Kaneko S, Akaike A. (2006) Serofendic acid, a neuroprotective substance derived from fetal calf serum, inhibits mitochondrial membrane depolarization and caspase-3 activation. *Eur J Pharmacol*, 542, 69.
6. Fennell M, Chan H, Wood A. (2006) Multiparameter measurement of caspase 3 activation and apoptotic cell death in NT2 neuronal precursor cells using high-content analysis. *J Biomol Screen*, 11, 296.
7. Wu X, Simone J, Hewgill D, Siegel R, Lipsky PE, He L. (2006) Measurement of two caspase activities simultaneously in living cells by a novel dual FRET fluorescent indicator probe. *Cytometry A*, 69, 477.
8. Jiang DJ, Jia SJ, Dai Z, Li YJ. (2006) Asymmetric dimethylarginine induces apoptosis via p38 MAPK/caspase-3-dependent signaling pathway in endothelial cells. *J Mol Cell Cardiol*, 40, 529.

Disclaimer: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.