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Caspase-3/7 Substrate Z-DEVD-R110

Table 1. Contents and Storage Information

Catalog No.	Material	Amount	Storage	Stability
A042	Z-DEVD-R110	5 mg	-20 °C Protect from light	The product is stable for 1 year when stored as directed.

Introduction

Caspases are key components of the apoptotic machinery of cells, participating in an enzyme cascade that results in cellular disassembly. The recognition site for caspases is marked by three to four amino acids followed by an aspartic acid residue, with the cleavage occurring after the aspartate. These proteases are typically synthesized as inactive precursors (procaspases); inhibitor release or cofactor binding activates the enzymatic activity through cleavage at internal aspartates through autocatalysis or by the action of another protease.

The caspase-3/7 substrate is a bisamide derivative of the fluorophore rhodamine 110 (R110). Peptides are covalently linked to each of the amino groups of rhodamine 110 (R110), thereby suppressing both the visible absorption and fluorescence of the dye. Upon enzymatic cleavage, the nonfluorescent bisamide substrate is converted in a two-step process, first to the fluorescent monoamide and then to the even more fluorescent R110. The R110 cleavage product has spectral properties similar to those of fluorescein and a large extinction coefficient, thus providing ease of use under standard fluorescein filter set-ups and excellent signal to background ratios. The substrate can be used to continuously measure enzyme activity in cell extracts and purified enzyme preparations using a fluorometer or fluorescence microplate reader.

Substrate Specifications:

- Substrate name: Z-DEVD-R110
- Molecular weight: 1515.5
- Peptide sequence: DEVD
- Useful to measure: Caspase-3, -6, -7, -8, -10
- Ex/Em of R110 product: 498/521 nm

Application

Assays with the caspase substrate are typically performed at room temperature in 10 mM HEPES or PIPES buffer, pH 7.2-7.4, 2 mM EDTA and 0.1% CHAPS or other detergent to promote cell lysis. Cells may also be lysed prior to assaying for caspase activity. The substrate stock solution in DMSO or DMF is diluted into the assay buffer to the desired working concentration (often ~100 µM) just prior to initiating the reaction by addition of the enzyme preparation or cell lysate.