Rhodamine 110–Based Caspase Substrates

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Abs/Emof R110 reaction product:

498/521 nm

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.[1]

Molecular Probes' rhodamine 110–based caspase substrates[2] are bisamide derivatives of the fluorophore; 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.

In addition to our caspase-selective substrates, we offer a generic bis-L-aspartic acid amide of R110 (D_2-R110, R-22122) and a substrate for granzyme B (Table 1). Granzyme B is a serine protease contained within cytotoxic T lymphocytes and natural killer cells that is thought to induce apoptosis in target cells by activating caspases and causing mitochondrial cytochrome *c* release.[3]

Materials

All R110 substrates are supplied as lyophilized powder and should be stored desiccated at ≤–20°C. The molecular weight is indicated on the product label. A stock solution of 10–20 mM may be prepared in DMSO. Slight vortexing or sonication of about 2 minutes may be required to fully solubilize the substrate. The maximum solubility is close to 50 mM in DMSO. This solution can be stored desiccated at 4°C or below for at least three months.

Application

Assays with the caspase and granzyme B substrates 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 assying 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. The D_2-R110 caspase substrate is somewhat more cell permeant and therefore may be used for cellular imaging or other assay conditions where cell lysis is not desirable.

Table 1. Molecular Probes' selection of R110-based caspase substrates.

Peptide Sequence	Target for Caspases *	Catalog Number
YVAD	1, 4	R33750
VDVAD	2	R33755
DEVD †	3, 6, 7, 8, 10	R22120 ‡
VEID	6	R33754
IETD	8, Granzyme B	R22125 R22126 ‡
LEHD	9, 4, 5	R33751
LEED	13	R33753
AAD	Granzyme B	R33752
Aspartic acid	Generic	R22122

* Caspase substrates can often be cleaved by multiple enzymes. The caspase most often associated with the given peptide sequence is listed first. † The DEVD substrate is also available as part of Caspase-3 Assay Kits E13184 and R35100. ‡ Bulk packaging for high-throughput screening.

References

1. Science 281, 1312 (1998); **2.** Symmetric rhodamine 110–based substrates are covered by US 4,557,862 or US 4,640,893 and are sold for nonclinical, research and developmental purposes only; **3.** J Biol Chem 276, 6974 (2001); **4.** Biochemistry 38, 13906 (1999).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
R22120	rhodamine 110, bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide) (Z-DEVD-R110) *bulk packaging*	20 mg
R22122	rhodamine 110, bis-(L-aspartic acid amide), trifluoroacetic acid salt	1 mg
R22125	rhodamine 110, bis-(N-CBZ-L-isoleucyl-L-glutamyl-L-threonyl-L-aspartic acid amide) (Z-IETD-R110)	2 mg
R22126	rhodamine 110, bis-(N-CBZ-L-isoleucyl-L-glutamyl-L-threonyl-L-aspartic acid amide) (Z-IETD-R110) *bulk packaging*	20 mg
R33750	rhodamine 110, bis-(N-CBZ-L-tyrosinyl-L-valyl-L-alanyl-L-aspartic acid amide) (Z-YVAD-R110)	2 mg
R33751	rhodamine 110, bis-(N-CBZ-L-leucyl-L-glutamyl-L-histidinyl-L-aspartic acid amide) (Z-LEHD-R110)	2 mg
R33752	rhodamine 110, bis-(N-CBZ-L-alanyl-L-alanyl-L-aspartic acid amide) (Z-AAD-R110)	2 mg
R33753	rhodamine 110, bis-(N-CBZ-L-leucyl-L-glutamyl-L-glutamyl-L-aspartic acid amide) (Z-LEED-R110)	2 mg
R33754	rhodamine 110, bis-(N-CBZ-L-valyl-L-glutamyl-L-isoleucyl-L-aspartic acid amide) (Z-VEID-R110)	2 mg
R33755	rhodamine 110, bis-(N-CBZ-L-valyl-L-aspartyl-L-valyl-L-alanyl-L-aspartic acid amide) (Z-VDVAD-R110)	2 mg

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