



Caspase-9 LEHD-R110 Fluorometric HTS Assay Kit

Catalog Number: 30015

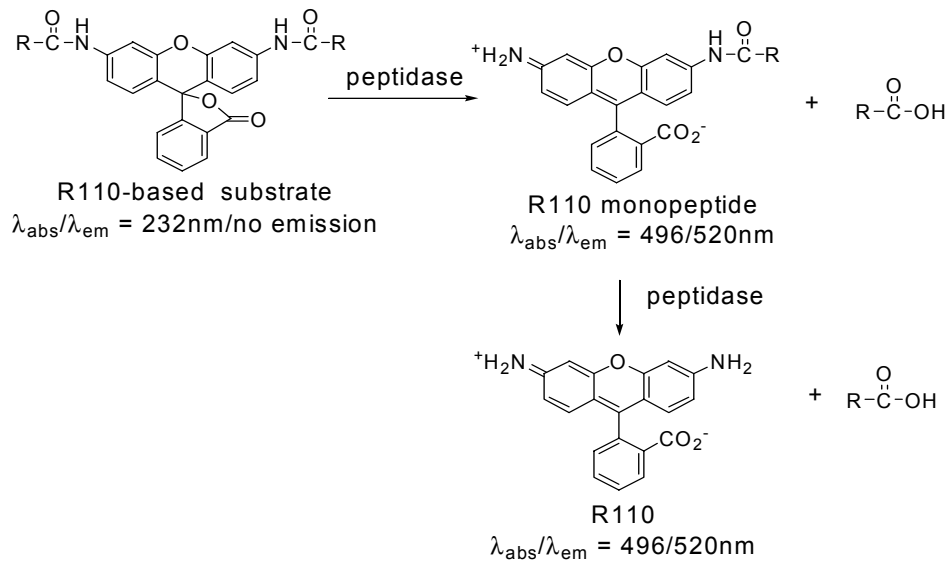
Contact Information

Address: Biotium, Inc.
3423 Investment Blvd. Suite 8
Hayward, CA 94545
USA

Telephone: (510) 265-1027
Fax: (510) 265-1352
Email: btinfo@biotium.com
Website: www.biotium.com

Description

Caspase-9 is an upstream caspase for mitochondrion-specific apoptosis pathway and is activated via the mitochondrial release of cytochrome c to the cytosol^(1,2). Caspase-9 LEHD-R110 HTS Assay Kit provides a single-step homogenous assay specifically designed for HTS-based detection. The fluorogenic substrate (Z-LEHD)₂-R110 contains two LEHD tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps. Cleavage of the first LEHD peptide results in the mono-peptide Z-LEHD-R110 intermediate, which has absorption and emission wavelengths similar to those of R110 ($\lambda_{abs}/\lambda_{em}=496/520$ nm), but has only about 10% the fluorescence of the latter⁽³⁻⁴⁾. Hydrolysis of the second LEHD peptide releases the dye R110, leading to a substantial fluorescence increase.



The assay kit includes LEHD-CHO, which is a Caspase-9 inhibitor and can be used as a negative control. Also, R110 is provided in the kit for generating a standard curve, which can be used for quantifying Caspase-9 activity.

Kit Components

1mL (#30015-1)	10mL (#30015-2)	100mL (#30015-3)	
1mL	10mL	100mL	Cell Lysis/Assay Buffer
20 L	200 L	2mL	
5 L	20 L	100 L	
1mL	1mL	1mL	
			Enzyme Substrate
			(Z-LEHD)₂-R110 (2.5mM)
			Enzyme Inhibitor
			Ac-LEHD-CHO (5mM)
			R110 (80 M)

Storage Condition

Caspase-9 LEHD-R110 Fluorometric HTS Assay Kit should be stored at -20°C or below. The components of the kit are stable at -20°C for three months and at -70°C for up to six months. Avoid frequent freeze-thaw cycles.

Features

HTS-compatible: Single-step homogenous assay specifically designed for HTS-based detection.

Fast: Fast enzyme kinetics.

Sensitive: The enzymatic reaction forms an intensely green fluorescent rhodamine 110 (R110) product. The long wavelength of R110 excitation and emission minimize cellular autofluorescence.

Assay for Detection of Caspase-9 Activity in Cell Culture

A. General Considerations

We recommend performing three control reactions:

- 1) Negative control on uninduced cells.
- 2) Control on induced cells treated with Caspase-9 inhibitor.
- 3) Positive control for Caspase-9 induction.

B. Preparation of Caspase-9 Detection Buffer

Depending on the required volume of Caspase-9 Detection Buffer, mix the Enzyme Substrate (Z-LEHD)₂-R110 (2.5mM) with the Cell Lysis/Assay Buffer in a 20 μ L to 1mL ratio to derive Caspase-9 Detection Buffer.

C. Assay Procedure

1. Induce apoptosis in cells by desired methods. Remember to incubate concurrent culture without induction.

2. For suspension cells:

Count cells and aliquot equal number of cells into each well in a 96-well plate or 384-well plate. It is recommended to use 500-50,000 cells per sample in the cell medium whose volume is less than one tenth of Caspase-9 Detection Buffer. For example, cells should be in 10 μ L or less medium in each well if 100 μ L Caspase-9 Detection Buffer will be used for each assay.

For attached cells:

Aspirate cell medium in each well of the plate where cells have been plated and induced.

3. Add Caspase-9 Detection Buffer directly into each well. 100 μ L is recommended for each well of a 96-well plate.

4. **[Optional]** To verify that the signal detected by the kit is due to Caspase-9 activity, incubate an induced sample with Caspase-9 inhibitor before adding substrate. This can be accomplished by adding 50 μ L of Cell Lysis/Assay Buffer and 1 μ L of Enzyme Inhibitor Ac-LEHD-CHO (5mM) to the cell suspension in a well of a 96-well plate. Incubate on ice for 30 min or RT for 15 min followed by adding 50 μ L of Cell Lysis/Assay Buffer and 2 μ L Enzyme Substrate (Z-LEHD)₂-R110 (2.5mM).

5. Incubate at 37°C for 30 min to 1hr (or up to 3 hours maximum) in an incubator.

8. Read in a fluorometer with 470 nm excitation filter and 520 nm emission filter for optimal sensitivity.

9. Use R110 if necessary for generating a standard curve to calculate amount of substrate conversion.

References

1. Kuida K. Caspase-9. Int J Biochem Cell Biol. 2000 Feb;32(2):121-4.
2. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol. 1999;15:269-90.

3. An S, Zheng Y, Bleu T. Sphingosine 1-phosphate-induced cell proliferation, survival, and related signaling events mediated by G protein-coupled receptors Edg3 and Edg5. *J Biol Chem.* 2000 Jan 7;275(1):288-96.
4. Hug H, Los M, Hirt W, Debatin KM. Rhodamine 110-linked amino acids and peptides as substrates to measure caspase activity upon apoptosis induction in intact cells. *Biochemistry.* 1999 Oct 19;38(42):13906-11.