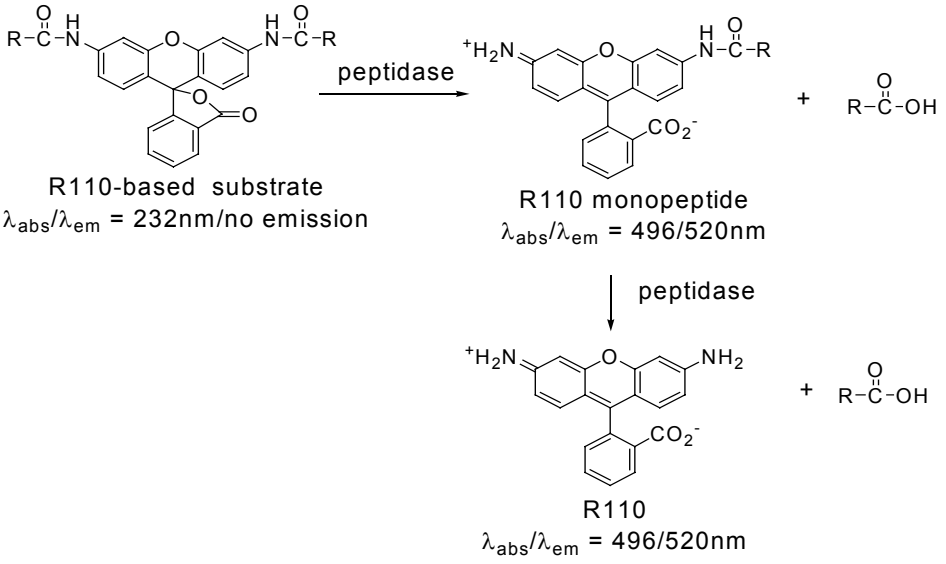
DATA SHEET

Caspase-3 DEVD-R110
Fluorometric HTS Assay Kit

Catalog Number: 30009

Description

Caspase-3 is an active cell-death protease involved in the execution phase of apoptosis, during which cells undergo morphological changes such as DNA fragmentation, chromatin condensation, and apoptotic body formation [1,2]. Caspase-3 DEVD-R110 Fluorometric HTS Assay Kit provides a single-step homogenous assay specifically designed for HTS-based detection. The fluorogenic substrate $(Ac\text{-}DEVD)_2$-R110 contains two DEVD tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps. Cleavage of the first DEVD peptide results in the monopeptide Ac-DEVD-R110 intermediate, which has absorption and emission wavelengths similar to those of R110 ($\lambda_{abs}/\lambda_{abs}$=496/520 nm), but has only about 10% the fluorescence of the latter [3-4]. Hydrolysis of the second DEVD peptide releases the dye R110, leading to a substantial fluorescence increase.



The assay kit includes DEVD-CHO, which is a caspase-3 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-3 activity.

Kit Components

1mL (#30009-1)	**10mL** (#30009-2)	**100mL** (#30009-3)	
1mL	10mL	100mL	**Cell Lysis/Assay Buffer**
50uL	500uL	5mL	**Enzyme Substrate $(Ac\text{-}DEVD)_2$-R110** (2mM)
5uL	20uL	100uL	**Enzyme Inhibitor Ac-DEVD-CHO** (5mM)
1mL	1mL	1mL	**R110** (80μM)

Storage Condition

Caspase-3 DEVD-R110 Fluorometric and Colorimetric Assay Kit should be stored at –20°C or below. The components of the kit are stable at –20°C for 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-3 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-3 inhibitor.
3) Positive control for Caspase-3 induction.

B. Preparation of Caspase-3 Detection Buffer

Depending on the required volume of Caspase-3 Detection Buffer, mix the Enzyme Substrate (Ac-DEVD)$_2$-R110 (2mM) with the Cell Lysis/Assay Buffer in a 50µL to 1mL ratio to derive Caspase-3 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 equal to the volume of Caspase-3 Detection Buffer to be added. For example, cells should be in 100µL medium in each well if 100µL Caspase-3 Detection Buffer will be used for each assay.

3. Add Caspase-3 Detection Buffer in equal volume to cell medium directly into each well.

4. **[Optional]** To verify that the signal detected by the kit is due to Caspase-3 activity, incubate an induced sample with caspase-3 inhibitor before adding substrate. This can be accomplished by adding 100µL of Cell Lysis/Assay Buffer and 2µL of Enzyme Inhibitor Ac-DEVD-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 5µL Enzyme Substrate (Ac-DEVD)$_2$-R110 (2mM).

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

6. Read on a fluorometer with 470 nm excitation filter and 520 nm emission filter for optimal sensitivity.

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

References

1. Porter AG, Janicke RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999 Feb;6(2):99-104.
2. Zou H, Li Y, Liu X, Wang X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem. 1999 Apr 23;274(17):11549-56.
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.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium