



Caspase-8 IETD-R110 Fluorometric HTS Assay Kit.

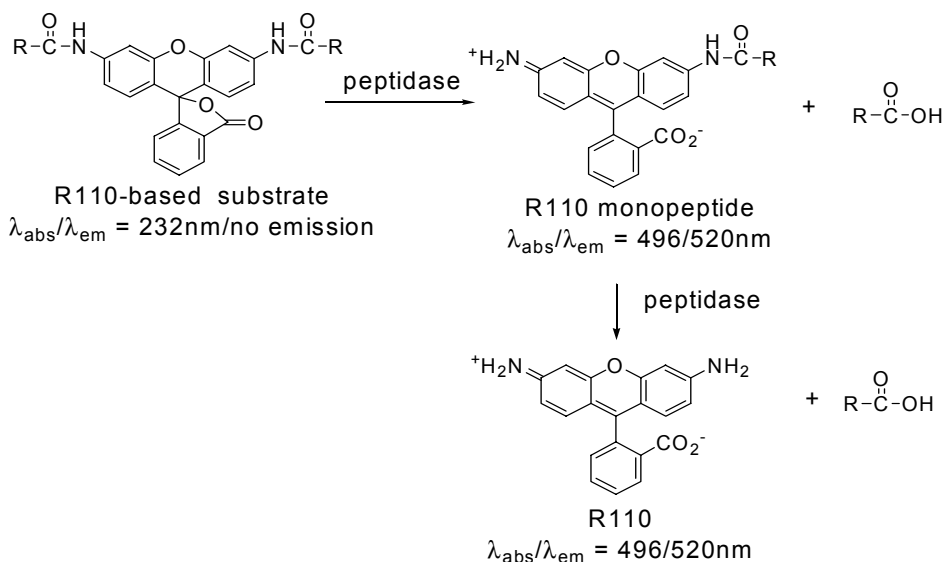
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I. Description

Caspase-8 is the most upstream caspase in the CD95/Fas apoptotic pathway and is activated by the signaling pathway for CD95/Fas and TNF (1, 2). Caspase-8 IETD-R110 HTS Assay Kit provides a single-step homogenous assay specifically designed for HTS-based detection assay. The fluorogenic substrate (Ac-IETD)₂-R110 contains two IETD tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps. Cleavage of the first IETD peptide results in the mono-peptide Ac-IETD-R110 intermediate, which has absorption and emission wavelengths similar to those of R110 ($\lambda_{\text{abs}}/\lambda_{\text{em}}=496/520$ nm) but has only about 10% of the fluorescence of the latter (3-4). Hydrolysis of the second IETD peptide releases the dye R110, leading to a substantial fluorescence increase.



The assay kit includes IETD-CHO, which is a Caspase-8 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-8 activity.

II. Kit Components

1 mL (30012-1)	10 mL (30012-2)	100 mL (30012-3)	
1 mL	10 mL	100 mL	Cell Lysis/Assay Buffer
50 uL	500 uL	5 mL	Enzyme Substrate (Ac-IETD)₂-R110 (2 mM)
5 uL	20 uL	100uL	Enzyme Inhibitor Ac-IETD-CHO (5 mM)
1 mL	1 mL	1 mL	R110 (80 uM)

III. Storage Condition

Caspase-8 IETD-R110 Fluorometric HTS 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.

IV. Features

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

Fast: Fast enzyme kinetics.

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

V. Assay for Detection of Caspase-8 Activity in Cell Culture

A. General Considerations

We recommend performing three control reactions:

- 1) a negative control on uninduced cells.
- 2) a control on induced cells treated with Caspase-8 inhibitor.
- 3) a positive control for Caspase-8 induction.

B. Preparation of Caspase-8 Detection Buffer

Depending on the required volume of Caspase-8 Detection Buffer, mix the Enzyme Substrate (Ac-IETD)₂-R110 (2 mM) with the Cell Lysis/Assay Buffer in 50 uL to 1 mL ratio to derive Caspase-8 Detection Buffer.

C. Assay Procedure

1. Induce apoptosis in cells by desired methods. Remember to incubate concurrent culture without induction.
2. 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-8 Detection Buffer to be added. For example, cells should be in 100 μ L medium in each well if 100 μ L Caspase-8 Detection Buffer will be used for each assay.
3. Add Caspase-8 Detection Buffer with equal volume to cell medium directly into each well.
4. **[Optional]** To verify that the signal detected by the kit is due to Caspase-8 activity, incubate an induced sample with Caspase-8 inhibitor before adding substrate. This can be accomplished by adding 100 μ L Cell Lysis/Assay Buffer and 2 μ L of Enzyme Inhibitor Ac-IETD-CHO (5 mM) to cell suspension in a well of a 96-well plate. Incubate on ice for 30 min or RT for 15 min followed by adding 5 μ L Enzyme Substrate (Ac-IETD)₂-R110 (2 mM).
5. Incubate at 37°C from 30 min-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.

VI. References

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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.

