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Product Information

Caspase-3 DEVD-R110 Fluorometric HTS Assay Kit

Catalog Number: 30009-1, 30009-2, 30009-3

Unit Size:
30009-1: 1 mL (10 assays)
30009-2: 10 mL (100 assays)
30009-3: 100 mL (1000 assays)
Number of assays based on 96-well format

Kit Contents

Component	30009-1	30009-2	30009-3
Cell Lysis/Assay Buffer	1 mL 99920	10 mL 99921	100 mL 99922
Enzyme Substrate $(Ac\text{-}DEVD)_2$-R110 (2 mM)	50 uL 30009-1A	500 uL 30009-2A	5 mL 30009-3A
Enzyme Inhibitor Ac-DEVD-CHO (5 mM)	5 uL 99926	20 uL 99927	100 uL 30009-3B
R110 (80 uM)	1 mL 99906	1 mL 99906	1 mL 99906

Storage and Handling
Store at –20°C and avoid multiple freeze-thaw cycles. The kit is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties: Ex/Em: 496/520 nm

Product 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). Caspase-3 DEVD-R110 Fluorometric HTS Assay Kit provides a single-step homogenous assay specifically designed for high throughput screening (HTS) for caspase-3 activity. 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 (Abs/Em 496/520 nm), but has only about 10% the fluorescence of the latter (2). Hydrolysis of the second DEVD peptide releases the green fluorescent dye R110, leading to a substantial fluorescence increase.

The assay kit includes the competitive caspase-3 inhibitor Ac-DEVD-CHO for use 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.

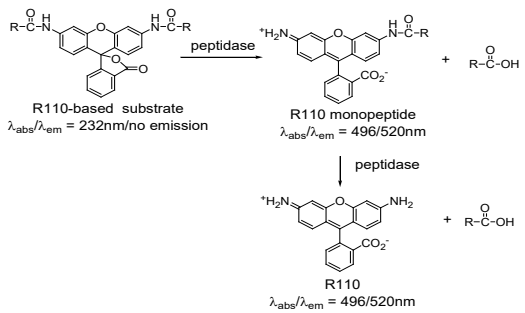

Figure 1. Two-step cleavage of R110-based substrates by peptidases to release green fluorescent R110 dye.

Protocol

The following protocol is designed for use in 96-well plates with a total assay volume of 200 uL per well. Volumes can be scaled proportionally as needed.

A. General Considerations

We recommend performing three control reactions:
1) Negative control using untreated cells
2) Positive control using cells treated with an apoptosis inducing agent
3) Inhibitor control using induced cells and Caspase-3 inhibitor.

B. Preparation of Assay Buffer

Prepare 100 uL assay buffer per sample. Add Enzyme Substrate $(Ac\text{-}DEVD)_2$-R110 (2 mM) to Cell Lysis/Assay Buffer at a ratio of 50 uL Substrate per 1 mL Buffer and mix well.

C. Assay Procedure

1. Plate cells in 100 uL culture medium per well of a black 96-well plate. For suspension cells, the recommended cell density is 500-50,000 cells in 100 uL medium per well.
2. Induce apoptosis in cells by desired methods. Remember to include untreated wells as controls.
3. Add 100 uL of assay buffer (from Step B) directly to 100 uL cells in culture medium in each well.
   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. Mix 100 uL of Cell Lysis/Assay Buffer and 2 uL of Enzyme Inhibitor Ac-DEVD-CHO (5 mM) for each inhibitor control well. Add 100 uL per well of cells. Incubate on ice for 30 minutes or room temperature for 15 minutes. Add 5 uL Enzyme Substrate $(Ac\text{-}DEVD)_2$-R110 (2 mM) and mix well.
4. Incubate plate at 37°C for 30-60 minutes (up to 3 hours maximum).
5. Measure fluorescence with 470 nm excitation and 520 nm emission.
   Optional: R110 can be used to generate a standard curve to calculate the amount of substrate conversion in experimental samples.

D. R110 Reference Standard (Optional)

1. Dilute R110 (80 uM) to 20 uM in Cell Lysis Buffer. Perform 1:2 serial dilutions to obtain concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 uM R110. Use Cell Lysis Buffer for the 0 uM (blank) sample. Add 100 uL/well of the serially diluted R110 solutions from 20 uM to 0 uM into a 96-well plate.
2. Measure the fluorescence intensity of the standards at Ex/Em=470 nm/520 nm. Subtract the fluorescence reading from the blank (0 uM R110) from each fluorescence value to calculate relative fluorescence units (RFU).
3. Plot RFU versus R110 concentration to generate a standard curve.

   Note: The kinetics of fluorescence generation due to substrate cleavage are not linear because the two-step cleavage of the substrate generates an intermediate and an end-product with different fluorescence intensities (see Product Description). Therefore, the R110 standard can be used to quantitate the amount of R110 generated at the endpoint of the assay, but cannot be used for kinetic studies.

References

1. Porter AG, Janicke RU. (1999) Emerging roles of caspase-3 in apoptosis. Cell Death Differ 6(2):99-104.
2. Hug H, Los M, Hirt W, Debatin KM. (1999) Rhodamine 110-linked amino acids and peptides as substrates to measure caspase activity upon apoptosis induction in intact cells. Biochemistry 38(42):13906-11.

Related Products

Catalog number	Product
30008	Caspase-3 DEVD-R110 Fluorometric & Colorimetric Assay Kit
30029	NucView 488 Caspase-3 Substrate Kit for Live Cells
30067	Dual Apoptosis Assay Kit with NucView 488 & CF594-Annexin V
30062	NucView 488 and MitoView 633 Apoptosis Kit
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30001	JC-1 Mitochondrial Membrane Detection Kit
70055	MitoView 633 mitochondrial membrane potential dye
30063	CF488A TUNEL Assay Apoptosis Detection Kit
30064	CF594 TUNEL Assay Apoptosis Detection Kit

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