Product Information

Caspase-3 DEVD-R110 Fluorometric and Colorimetric Assay Kit

Catalog Number: 30008-1, 30008-2

Unit Size:
30008-1: 25 assays
30008-2: 100 assays
Assays based on 96-well format

Kit Contents

Component	30008-1	30008-2
Cell Lysis Buffer	30 mL 99917	100 mL 99918
Assay Buffer	1 X 1.25 mL 99919	4 X 1.25 mL 99919
Enzyme Substrate $(Ac\text{-}DEVD)_2$-R110 (1 mM)	125 uL 30008-1A	500 uL 30008-2A
Enzyme Inhibitor Ac-DEVD-CHO (5 mM)	5 uL 99926	20 uL 99927
R110 (80 uM)	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.

Absorption/Emission: 496/520 nm (after cleavage)

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.

Although fluorometric detection of R110 is preferred due to superior sensitivity, absorbance-based measurements also can be used. In fact, the extinction coefficient of R110 is 10 times higher than that of p-nitroaniline (pNA), a dye commonly used in chromogenic substrates. Therefore, R110-based substrates are significantly more sensitive than pNA-based substrates, even by colorimetric detection. The intensity of the fluorescent or colorimetric signal generated from the assay is proportional to the caspase-3 activity present in the sample.

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.

Biotium also offers the Caspase-3 DEVD-R110 Fluorometric HTS Assay Kit (catalog number 30009), a single step homogeneous assay for high throughput screening (HTS) using fluorescence-based measurement.

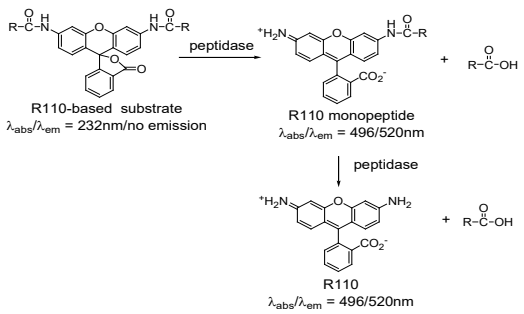

Figure 1. Two-step cleavage of R110-based substrates by peptidases to release green fluorescent R110 dye. R represents the peptide substrate (DEVD for Caspase-3 substrate).

General Considerations

The following are general considerations for using Caspase-3 DEVD-R110 Fluorometric and Colorimetric Assay Kit.

- This kit may be used for fluorescence-based or absorbance-based detection.
- The following protocols can be performed in 96-well microplates or microcentrifuge tubes.
- For fluorescence microplate readers, we recommend using black plates to reduce background.
- For absorbance microplate readers, clear-bottom plates must be used.
- We recommend performing the following controls:
  a. Negative control using untreated cells
  b. Positive control using cells induced to undergo apoptosis using a method validated for your cell type
  c. Inhibitor control using apoptosis-induced cells and caspase-3 inhibitor

Assay Procedure

The following protocol is designed for use in 96-well plates with a total assay volume of 100 uL per well. Volumes can be scaled proportionally as needed.

4. Plate adherent cells in appropriate 96-well plates. Suspension cells can be plated in flasks or plates.

5. Induce apoptosis in cells by desired methods. Remember to include untreated wells as controls.

6. Cell lysis procedure:

   For suspension cells:

   a. Aliquot equal numbers of cells into microcentrifuge tubes or wells of a 96-well plate. 500-1,000,000 cells per sample can be used for fluorometric detection (10,000-100,000 cells is optimal for Jurkat cells), while 1,000,000 cells per sample is required for colorimetric detection.

   b. Centrifuge cells at 400 xg for 5 minutes and aspirate supernatant. For fluorometric detection, it is not necessary to centrifuge cells as long as cells are suspended in less than 10 uL medium.

      Note: For fluorometric detection, if the volume of cell suspension is less than 10 uL, the cells can be added directly to lysis buffer without centrifuging.

      Optional: After this step, you may freeze the cell pellets at -70°C to assay at a later time.

   c. Resuspend the cell pellets in 50 uL of chilled Cell Lysis Buffer.

   For adherent cells:

   a. Aspirate culture medium from each well of the 96-well plate. Add 50 uL chilled Cell Lysis Buffer per well.

2. Incubate cells in Lysis Buffer on ice for 10 minutes.

3. For colorimetric detection only: Centrifuge the cell lysate directly in the 96-well plate or transfer cell lysates into fresh microcentrifuge tubes and centrifuge at maximum speed for 5 minutes at 4°C to pellet insoluble cell debris. Transfer the supernatants to a fresh 96-well plate or microcentrifuge tubes.

   Note: For fluorometric detection, it is not necessary to centrifuge lysates. All steps can be performed in the same plate.

4. Add 50 uL of Assay Buffer to each sample and mix 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. Add 1 uL of Enzyme Inhibitor Ac-DEVD-CHO (5 mM) to each inhibitor control sample. Incubate on ice for 30 minutes or room temperature for 15 minutes along with the other samples.

5. Add 5 uL of 1 mM Enzyme Substrate to each sample and mix well. Incubate samples at 37°C for 30-60 minutes (up to 3 hours maximum).

6. Measure fluorescence with 470 nm excitation and 520 nm emission. For colorimetric measurement, measure absorbance at 495 nm.

Optional: R110 Reference Standard

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 or absorbance of the standards. Subtract the reading from the blank (0 uM R110) from each value to calculate relative fluorescence or absorbance.

3. Plot relative fluorescence or absorbance 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. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999 Feb;6(2):99-104.
2. 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.

Related Products

Catalog number	Product
30009	Caspase-3 DEVD-R110 Fluorometric HTS 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	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
29002... 29083	Annexin V Conjugates
29003R... 29069R	Annexin V CF® Dye Conjugates, Azie-Free, Lyophilized
29006...29082	Annexin V Near IR CF® Dye Conjugates
70005	TMRE, 2 mM in DMSO
70016	TMRE (Tetramethylrhodamine ehtyl ester, perchlorate)
70017	TMRM (Tetramethylrhodamine methyl ester, perchlorate)
70011	JC-1 (chloride salt)

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