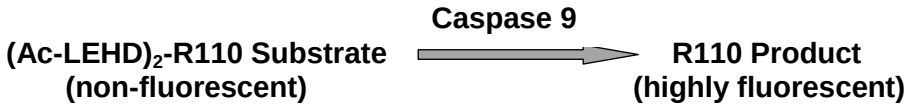
Elite™ Caspase 9 Assay Kit (Green Fluorescence)

CATALOG NUMBER: CA-C149, 2 plates

Description

Caspases play important roles in apoptosis and cell signaling. Caspase 9 is a member of the CED-3 subfamily. Activated Caspase-9 cleaves downstream caspases such as caspase-3, -6 and -7, initiating the caspase cascade. It is essential for apoptosis during normal development of the central nervous system.

Caspase 9 is proven to have selectivity for the peptide sequence Leu-Glu-His-Asp (LEHD). This kit uses $(Ac\text{-}LEHD)_2$-R110 as a fluorogenic indicator for caspase 9 activity. Cleavage of R110 peptides by caspase 9 generates strongly fluorescent rhodamine 110 (R110) which is monitored at the emission between 520 nm and 530nm with the excitation between 480 nm and 500 nm. The kit provides all the essential components. The assay is robust and can be readily adapted for high throughput screening. It can be used to either quantify the activated caspase 9 activities in apoptotic cells or screen the caspase 9 inhibitors. Quite a few labs have used this kit for high throughput screenings.



Features

- **Optimized Performance**: Optimal conditions for the detection of caspase 3 activity.
- **Continuous**: Easily adapted to automation without a separation step.
- **Convenient**: Include all essential assay components.
- **Non-radioactive**: No special requirement for waste disposal.

Kit Components

- **Component A**: Caspase 9 Substrate (200x stock solution), 2 vial (50 µl/vial)
- **Component B**: Assay Buffer, 20 ml

Storage

Keep **Component A** in dark at -20 °C and **Component B** at 4 °C.

Materials Required (but not supplied)

- 96 or 384-well microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- A fluorescence microplate reader: Capable of monitoring fluorescence intensity at Ex/Em = 490±10/520±10 nm.

Assay Protocol (for 96-well plate)

1. **Prepare Cells:**
   - 1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 cells/well/90 µl for a 96-well plate or 5,000 cells/well/20 µl for a 384-well plate.
   - 1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 80,000 cells/well/90 µl for a 96-well poly-D lysine plate or 20,000 cells/well/20 µl

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for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Prepare caspase 9 assay loading solution:

2.1 Thaw both of the kit components to room temperature before use.

2.2 Prepare caspase 9 assay loading solution: Add 50 µL of Caspase 8 Substrate (**Component A**) into 10 ml of Assay Buffer (**Component B**), and mix them well.

Note: Aliquot and store unused Caspase 9 Substrate (**Component A**) and Assay Buffer (**Component B**) at -20 ^{o}C. Avoid repeated freeze/thaw cycles.

3. Run caspase 9 assay:

3.1. Treat cells by adding 10 µl of 10X test compounds (96-well plate) or 5 µl of 5X test compounds (384-plate) into PBS or desired buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

3.2. Incubate the cell plates in an incubator (5% CO_2, 37 ^{o}C) for a desired period of time (4 - 6 hours for Jurkat cells treated with camptothecin) to induce apoptosis.

3.3. Add 100 µl /well (96-well plate) or 25 µl /well (384-well plate) of caspase 9 assay loading solution (from step 2.2).

3.4. Incubate the plate at room temperature for at least 1 hour, kept from light.

Note: If desired, add 1 µl of the 1 mM Ac-LEHD-CHO Caspase 9 Inhibitor into selected samples 10 minutes before adding the assay loading solution at room temperature to confirm the inhibition of the Caspase 9 -like activities.

3.5. Centrifuge cell plates (especially for the non-adherent cells) at 800 rpm for 2 minutes with brake off.

3.6. Monitor the fluorescence increase at Ex/Em = 490/525 nm.

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with the cells. The background fluorescence of the blank wells varies depending upon the sources of the growth media or the microtiter plates.

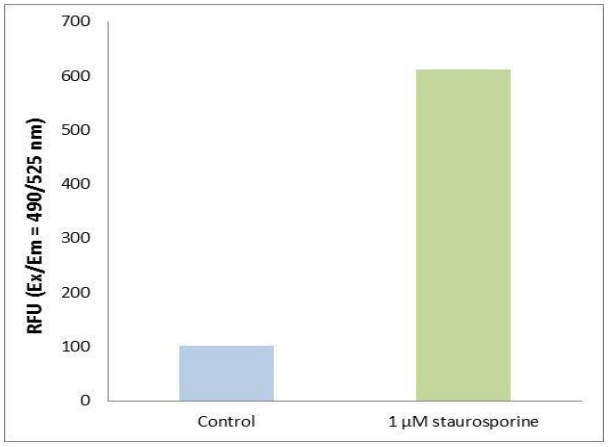

Figure 1. Detection of caspase 9 Activity in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells /well/90 µl in a black wall/clear bottom 96-well plate. The cells were treated with 1 µM of staurosporine for 5 hours while the untreated cells were used as control. The caspase 9 assay loading solution (100 µl/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 490/525 nm with a FlexStation™ microplate reader (Molecular Devices).

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