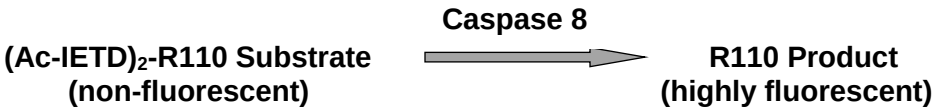
Elite™ Caspase 8 Assay Kit (Green Fluorescence)

CATALOG NUMBER: CA-C158, 2 plates

Description

Caspases play important roles in apoptosis and cell signaling. Caspase 8 is a caspase protein, encoded by the CASP8 gene. Caspase 8 also plays an important role in neurodegenerative diseases, such as Huntington disease.

Caspase 8 is proven to have substrate selectivity for the peptide sequence Ile-Glu-Thr-Asp (IETD). This kit uses $(Ac\text{-}IETD)_2$-R110 as a fluorogenic indicator for caspase 8 activity. Cleavage of rhodamine 110 (R110) peptides by caspase 8 generates strongly fluorescent R110 which is monitored at the emission between 520 nm and 530 nm with the excitation between 480 nm and 500 nm. This spectral feature makes the kit compatible with the FITC filter set. The kit provides all the essential components with an optimized assay protocol. The assay can be readily adapted for high throughput screenings. It can be used to either quantify the activated caspase 8 activities in apoptotic cells or screen the caspase 8 inhibitors.



Features

- **Optimized Performance**: Optimal conditions for the detection of caspase 8 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 8 Substrate (200x stock solution)	2 vial (50 µl/vial)
• **Component B:** Assay Buffer	20 ml

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

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.

Shelf Life

All reagents are stable for at least 6 months after receipt when stored properly at the recommended conditions.

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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 200,000 cells/well/90 µl for a 96-well poly-D lysine plate or 50,000 cells/well/20 µl 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 8 assay loading solution:**

   2.1 Thaw both kit components to room temperature before use.

   2.2 Prepare caspase 8 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 1: Caspasae 8 assay loading solution is not stable, use promptly.

   Note 2: Aliquot and store unused Caspase 8 Substrate (**Component A**) and Assay Buffer (**Component B**) at -20 ^{o}C. Avoid repeated freeze/thaw cycles.

3. **Run caspase 8 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 a 5% CO_2, 37 °C incubator for a desired period (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 8 assay loading solution (from step 2.2).

   3.4. Incubate the plate with the assay loading solution at room temperature for 30 min to 1 hour, kept from light.

   Note: If desired, add 1 µl of the 1 mM Ac-DEVD-CHO Caspase 8 Inhibitor into selected samples 10 minutes before adding the assay loading solution at room temperature to confirm the inhibition of the Caspase 8 -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.

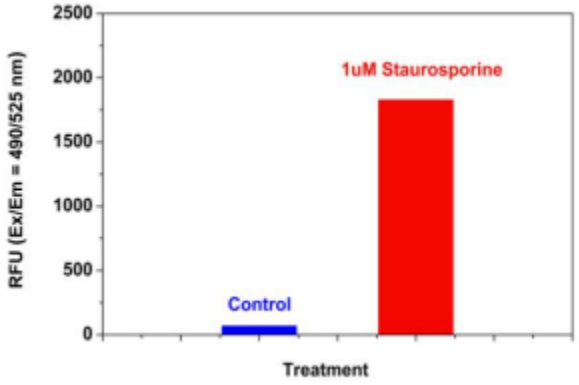

Figure 1. Detection of caspase 8 Activity in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells /well/90 µl in a black wall/clear bottom 96-well Costar plate. The cells were treated with or without 1 µM of staurosporine for 4 hours while the untreated cells were used as control. The caspase 8 assay loading solution (100 µl/well) was added and incubated at room temperature for 30 min. The fluorescence intensity was measured at Ex/Em = 490/525 nm with a FlexStation™ microplate reader (Molecular Devices).

References

1. N. A. Thornberry and Y. Lazebnik, Science 281, 1312-1316 (1998).
2. J. C. Reed, J.Clin.Oncol. 17, 2941-2953 (1999).
3. Y. A. Lazebnik, S. H. Kaufmann, S. Desnoyers, G. G. Poirier, W. C. Earnshaw, Nature 371, 346-347 (1994).
4. P. Villa, S. H. Kaufmann, W. C. Earnshaw, Trends Biochem.Sci. 22, 388-393 (1997).
5. Y. Liu et al., Anal.Biochem. 267, 331-335 (1999).
6. M. Sakaue, Y. Motoyama, K. Yamamoto, T. Shiba, T. Teshima, K.Chiba. Biochem Biophys Res Commun, 350, 878 (2006).
7. T. Kume, R. Taguchi, H. Katsuki, M. Akao, H. Sugimoto, S. Kaneko, A. Akaike. Eur J Pharmacol, 542, 69 (2006) (2006).
8. M. Fennell, H. Chan , A Wood. J Biomol Screen, 11, 296 (2006).
9. X. Wu, J. Simone, D. Hewgill, R. Siegel, PE. Lipsky, L. He. Cytometry A, 69, 477 (2006).

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