Technical Bulletin

Caspase-3 Assay Kit

Catalog Number MAK457

Product Description

Caspases are members of the aspartate-specific cysteinyl protease family that play a central role in apoptosis. Apoptosis is involved in a variety of physiological and pathological events, ranging from normal fetal development to diseases such as cancer, organ failure, and neurodegenerative diseases. Caspase-3 is key biomarker in the assessment of apoptosis and in understanding mechanism of apoptosis induction.

The Caspase-3 Assay Kit provides a convenient means to measure caspase-3 activity in biological samples. In the assay, a specific substrate (N-Ac-DEVD-AFC) is cleaved by active caspase-3, forming a highly fluorescent product. The fluorescence intensity (λ_{Ex} = 400 nm/λ_{Em} = 490 nm) generated by the reaction is proportional to the caspase-3 activity.

The kit is suitable for the relative determination caspase-3 activity in cell and tissue lysates and for and high throughput screening of apoptosis inducers and inhibitors.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 12 mL
  Catalog Number MAK457A
- Substrate 240 µL
  Catalog Number MAK457B
- DTT Solution 240 µL
  Catalog Number MAK457C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescent multiwell plate reader
- Sterile black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge capable of RCF ≥ 2,500 × *g*
- Cell culture incubator capable of 37 °C

Reagents Required for Alternative Assay Procedure

- HEPES
- (Catalog Number H3375 or equivalent)
- Sodium chloride (NaCl)
  (Catalog Number S9888 or equivalent)
- Triton™ X-100
  (Catalog Number X100 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

96-Well Assay Procedure

Note: The following procedure is for standard assays in a 96-well plate. If cells are cultured in plates other than a 96-well plate or flasks, it is necessary to prepare the cell lysate (See Alternative Assay Procedure).

All samples and controls should be run in duplicate.

Cell Culture

1. Seed 100 µL of 1,000 to 100,000 cells into wells of a sterile black clear-bottom 96-well plate. Prepare duplicate wells for use as Sample and corresponding Control.

   Note: The cell number to be used depends on the cell line being used.

2. Incubate overnight at 37 °C in a cell culture incubator.

Cell Treatment

1. Add 10 µL of test compounds (e.g., apoptosis inducers or inhibitors) at desired concentration to the appropriate Sample well(s).
2. To the corresponding Control well(s), add 10 µL of vehicle (i.e., medium in which the test compound is dissolved).
3. Incubate the cells for the desired period of time.

Caspase-3 Assay

1. Prior to assay, bring all reagents to room temperature and briefly centrifuge small tubes.
2. Mix enough reagents for the number of assays to be performed. For each well, prepare 104 µL of Working Reagent according to Table 1. The Working Reagent lyses cells and supports optimal caspase-3 activity.

Table 1.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	100 µL
Substrate	2 µL
DTT	2 µL

3. Remove cell culture media from assay wells. For adherent cells, aspirate the cell culture media from wells. For suspension cells, centrifuge cells for 5 minutes at 500 × *g*, before carefully remove media by aspiration.
4. Immediately add 100 µL of Working Reagent to each assay well.
5. Mix thoroughly by shaking the plate for 60 seconds at 100-200 rpm on a plate shaker.
6. Incubate the plate at 37 °C for 60 minutes in the dark.

Measurement

Measure the fluorescence intensity (RFU) at λ_{Ex} = 400 nm/λ_{Em} = 490 nm.

Alternative Assay Procedure

For cells not cultured in a 96-well plate, cell lysates are prepared separately and used for the caspase assay.

1. After cells have been treated with test compounds for the desired period of time, remove cell culture medium. For adherent cells simply aspirate the cell culture medium. For suspension cells centrifuge cells at 500 × *g* for 5 minutes and then aspirate the medium.
2. Lyse cells by adding 300 µL per 10^6 cells of 50 mM HEPES, pH 7.2, 100 mM NaCl, 0.5% (v/v) Triton X-100.
3. Shake the cell suspension for 30 minutes at 4 °C.
4. Centrifuge the cell suspension at 2,500 × *g* for 10 minutes at 4 °C.
5. Transfer the lysate supernatants to clean tubes. If not assayed on the same day, lysates can be stored at -80 °C for one month.
6. To assay Caspase-3 activity, add 50 µL of Sample lysates into separate wells of a black 96-well plate.
7. Immediately add 100 µL of Working Reagent to each assay well.
8. Incubate the plate at 37 °C for 60 minutes in the dark.
9. Measure the fluorescence intensity (RFU) at λ_{Ex} = 400 nm/λ_{Em} = 490 nm.

Results

Subtract the Sample fluorescence intensity values (RFU) from those of the Control wells. The ΔRFU values represent the relative caspase-3 activity.

If the caspase-3 activity is low, repeat the assay and increase the incubation time or use more cells.

Figure 1.
Time course of the induction of caspase-3 activity by staurosporine in RBL-2H3 and PANC-1 cells lines.

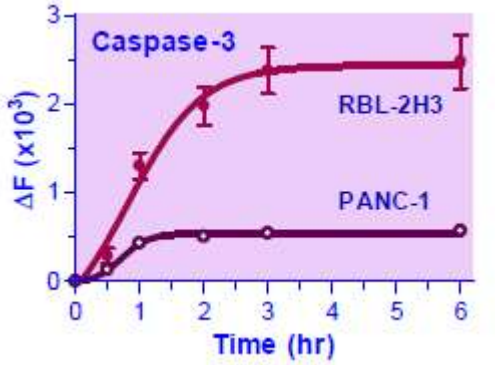
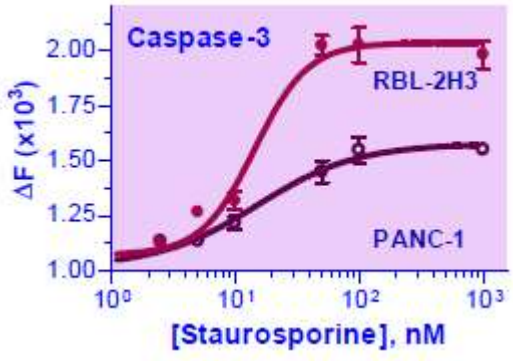


Figure 2.
Induction of caspase-3 by staurosporine in RBL-2H3 (EC_{50} = 14.2 nM) and PANC-1 (EC_{50} = 13.9 nM) cell lines



References

1. Hug, H., et al., Rhodamine 110-linked amino acids and peptides as substrates to measure caspase activity upon apoptosis induction in intact cells. *Biochemistry*, **38**, 13906-11 (1999).
2. Jones, J., et al., Development and application of a GFP-FRET intracellular caspase assay for drug screening. *J. Biomol. Screen.*, **5**, 307-18 (2000).
3. Wang, Z.-Q., et al., N-DEVD-N'-morpholinecarbonyl-rhodamine 110: novel caspase-3 fluorogenic substrates for cell-based apoptosis assay. *Bioorg. Med. Chem. Lett.*, 15, 2335-8 (2005).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.