

## QuantiFluo™ Caspase-3 Assay Kit (DCS3-100)

### Quantitative Fluorimetric Assay for Apoptosis at 400/490nm

#### 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.

BioAssay Systems' 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 ( $\lambda_{exc/em} = 400/490\text{nm}$ ) is proportional to the caspase-3 activity.

#### KEY FEATURES

**Safe.** Non-radioactive assay.

**Convenient and high-throughput.** Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

#### APPLICATIONS

**Determination** of caspase-3 activity in cell and tissue lysate.

HTS screening for apoptosis inducers and inhibitors.

#### KIT CONTENTS

**Assay Buffer:** 12 mL      **Substrate:** 240  $\mu\text{L}$   
**DTT Solution:** 240  $\mu\text{L}$

**Storage conditions:** This product is shipped at room temperature. Upon delivery, store all reagents at  $-20^{\circ}\text{C}$ . Shelf life of 12 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### 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, it is necessary to prepare the cell lysate (See General Considerations). Duplicate assays are recommended for controls and samples.*

1. **Cell culture.** Seed 100  $\mu\text{L}$  of 1,000 to 100,000 cells into wells of a sterile black clear-bottom 96-well plate (*Note: The cell number to be used depends on the cell line*). Incubate overnight at  $37^{\circ}\text{C}$  in a cell culture incubator.

2. **Cell treatment.** Add 10  $\mu\text{L}$  test compounds (e.g. apoptosis inducers or inhibitors) at desired concentration, and 10  $\mu\text{L}$  vehicle (medium in which the test compound is dissolved) as a control.

Incubate cells for a desired period of time.

3. **Caspase-3 Assay.** Prior to assay, bring all reagents to room temperature. Briefly centrifuge tubes. Prepare enough Working Reagent for all assay wells. For each well, mix 100  $\mu\text{L}$  Assay Buffer, 2  $\mu\text{L}$  Substrate and 2  $\mu\text{L}$  DTT.

Remove culture media from assay wells. For adherent cells, simply aspirate the culture media from wells. For suspension cells, centrifuge cells for 5 min at 500 x g, carefully remove media by aspiration.

Immediately add 100  $\mu\text{L}$  Working Reagent to each assay well. Mix the reagents completely by shaking the plate for 60 sec at 100-200 rpm on a plate shaker. *The Working Reagent lyses cells and supports optimal caspase-3 activity.*

Incubate plate at  $37^{\circ}\text{C}$  for 60 min in the dark.

4. **Read** fluorescence intensity at  $\lambda_{exc/em} = 400/490\text{ nm}$ .

*Note: If the caspase-3 activity is low, increase the incubation time, or use more cells.*

#### CALCULATION

Subtract the fluorescence intensity values from that of the control wells. The  $\Delta\text{F}$  values represent the relative caspase-3 activity.

#### GENERAL CONSIDERATIONS

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

After cells have been treated with test compounds for the desired period of treatment, remove culture medium. For adherent cells simply aspirate the culture medium. For suspension cells centrifuge cells at 500 x g for 5 min and aspirate the medium.

Lyse cells by adding 300  $\mu\text{L}$  per  $10^6$  cells of 50 mM Hepes (pH 7.2), 100 mM NaCl, 0.5 % (v/v) Triton X-100.

Shake the cell suspension on for 30 min at  $4^{\circ}\text{C}$ .

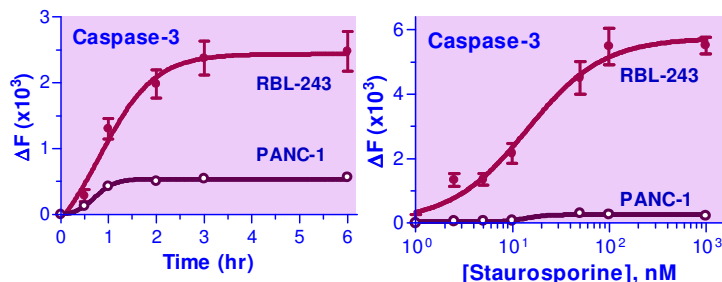
Centrifuge the cell suspension at 2,500 x g for 10 min at  $4^{\circ}\text{C}$ .

Transfer supernatants to clean tubes. If not assayed on the same day, lysates can be stored at  $-80^{\circ}\text{C}$  for one month.

To assay, add 50  $\mu\text{L}$  of sample lysates into separate wells of a black 96-well plate. Immediately add 100  $\mu\text{L}$  Working Reagent to each assay well. Incubate plate at  $37^{\circ}\text{C}$  for 60 min in the dark. Read fluorescence intensity  $\lambda_{exc/em} = 400/490\text{ nm}$ .

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, black flat bottom 96-well plates and plate reader.



*Time course of induction of caspase-3 activity by staurosporine in RBL-243 and PANC-1 cells lines.*

*Induction of caspase-3 by staurosporine in RBL-243 ( $\text{EC}_{50} = 14.2\text{ nM}$ ) and PANC-1 ( $\text{EC}_{50} = 13.9\text{ nM}$ ) cell lines.*

#### LITERATURE

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