**Signosis, Inc.**
Innovative Plate Assay Solutions

# Caspase Multiplex Assay Kit

**Catalog # EA-7021** **(For Research Use Only)**

## Introduction

Caspases are a group of cysteine-aspartic proteases that play an important role in apoptosis, inflammation, and cell differentiation. These proteases cleave proteins after aspartic residues and are activated through a cascade of proteolytic cleavages. Abnormalities in caspase activity has been linked to a variety of diseases. Mutations in caspases may lead to tumor growth, since these proteases normally induce cell death in abnormally growing cells. Conversely, over-activation of some caspases, such as caspase-3, may cause excessive programmed cell death, which is seen in several neurodegenerative diseases, including Alzheimer's disease. The critical role that caspases play in the development of different diseases has led to research on using caspases as a therapeutic target. Signosis's Caspase Multiplex Assay Kit provides a comprehensive solution for measuring key caspases in different sample types including blood, cells, and tissue. This kit can measure the activity of caspase-3, caspase-8, and caspase-9 by utilizing a unique fluorogenic substrate to detect each protease.

## Principle

Each caspase type interacts with a unique fluorogenic substrate that is specifically cleaved by each caspase variant. Once cleaved, the substrate releases a fluorescent dye which can be detected with a fluorescence plate reader. In this assay, the substrates for each caspase type have unique fluorogenic dyes with different emission spectra, which allows for multiplex detection of multiple caspase types in the same sample.

Caspase-3/7: Ac-DEVD-AMC (Ex/Em: 344nm/440nm)

Caspase-8: Ac-IETD-AFC (Ex/Em: 400nm/505nm)

Caspase-9: Ac-LEHD-R110 (Ex/Em: 500nm/522nm)

## Materials Required but Not Provided

- PBS
- 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring fluorescence

## Materials Provided

- Caspase 3/7 Substrate (-80ºC)
- Caspase 8 Substrate (-80ºC)
- Caspase 9 Substrate (-80ºC)

****Spin down small tubes before starting experiment. ****

**Plasma Sample Preparation**

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

**Cell Sample Preparation**

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration for each assay, if necessary.

**Tissue Sample Preparation**

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration for each assay, if necessary.

**Caspase Measurement**

1. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

| Component | Reaction Mix (per well/sample) |
|---|---|
| Caspase 3/7 Substrate | 2 µL |
| Caspase 8 Substrate | 2 µL |
| Caspase 9 Substrate | 2 µL |
| PBS | 54 µL |
| Total | 60 µL |

2. Add 60 µL of reaction mix to each well of the plate.
3. Add 60 µL of sample to each well with reaction mix and mix thoroughly.
4. Cover the plate and incubate at 37°C for 1-2 hours.
5. Measure the fluorescence of the plate in a fluorescence plate reader using the appropriate excitation/emission settings.