Signosis, Inc.
Innovative Plate Assay Solutions

Caspase-9 Assay Kit

Catalog # EA-7034 **(For Research Use Only)**

Introduction

Caspase-9 is an initiator caspase that functions as a central component of the intrinsic, or mitochondrial, pathway of apoptosis. In response to intracellular stress signals, cytochrome c is released from mitochondria and promotes the assembly of the apoptosome, a multiprotein complex that recruits and activates caspase-9. Once activated, caspase-9 triggers the apoptotic cascade by cleaving and activating executioner caspases, including caspase-3 and caspase-7. Through this role, caspase-9 acts as a key sensor and amplifier of internal damage signals leading to programmed cell death. Signosis's Caspase-9 Assay Kit utilizes a fluorogenic substrate that can measure caspase-9 in different sample types including blood, cells, and tissue.

Principle

The Caspase-9 Assay utilizes a unique fluorogenic substrate that is specifically cleaved by caspase-9. Once cleaved, the substrate produces a luminescent signal which can be detected with a fluorescence plate reader at Ex/Em: 400nm/505nm.

Caspase-9: Ac-LEHD-AFC (Ex/Em: 400nm/505nm)

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 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, 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, if necessary.

Caspase-9 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 9 Substrate	1 µL
PBS	49 µL
Total	50 µL

2. Add 50 µL of reaction mix to each well of the plate.
3. Add 50 µL of sample to each well with reaction mix and mix thoroughly.
4. Cover the plate and incubate at room temperature for 10 minutes away from light.
5. Measure the fluorescence of the plate in a fluorescence plate reader at Ex/Em: 400nm/505nm. Take measurements at multiple time intervals to monitor change in fluorescence.