Caspase 9 Assay Kit

ARG83412 Caspase 9 Assay Kit can be used to measure Caspase 9 in tissue extracts, cell lysate and other biological fluids.

Catalog number: ARG83412

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Caspase-9 is an enzyme that in humans is encoded by the CASP9 gene. It is an initiator caspase, critical to the apoptotic pathway found in many tissues. Caspase-9 homologs have been identified in all mammals for which they are known to exist, such as Mus musculus and Pan troglodytes.

PRINCIPLE OF THE ASSAY

Caspase 9 Assay Kit determined Caspase 9 based on the spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate. The increase in absorbance at 405 nm is directly proportional to the Caspase 9 activity.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (500 µmol/L)	1 ml	4°C
Assay Buffer I	2 X 30 ml	4°C
Assay Buffer II	0.6 ml	4°C
Substrate	1 vial (lyophilized)	-20°C
Reaction Buffer	6 ml	4°C
Reducing Agent	1 vial (lyophilized)	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Substrate and Reducing Agentat -20°C, other component at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

For cell and bacteria - Collect cell or bacteria into centrifuge tube, centrifuged at 600g 4 °C for 5 minutes, discard the supernatant, add 0.5 ml Assay Buffer I, 5 µl Assay Buffer II and 5 µl Reducing Agent, mix and keep it on ice for 10 minutes. Centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

For tissue - Weigh out 0.05 g tissue, homogenize with 0.5 ml Assay Buffer I, 5 µl Assay Buffer II and 5 µl Reducing Agent on ice for 10 minutes. Centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

REAGENT PREPARATION

- **Standard:** Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Substrate:** Reconstitute the Substrate with **1 ml** of **Reaction Buffer**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Reducing Agent:** Reconstitute the Substrate with **1 ml** of **distilled water**. Allow the Reducing Agent keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Reaction Buffer:** Add **0.1 ml Reducing Agent** before use. Make sure the Reaction Buffer mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **40 µl Sample** into Sample wells.
2. Control wells: Add **40 µl Assay Buffer I** into Control wells.
3. Add **50 µl** of **Reaction Buffer, 10 µl** of **Substrate** into Sample and Control wells.
4. Mix well. Incubate at **37°C** for **60 min**.
5. Standard wells: Add **100 µl** of **Standard Buffer** into Standard wells.
6. Mix well. Read the OD at **405 nm**.

Summary of Caspase 9 Assay Kit Procedure

Reagent	Sample	Control	Standard	Blank
Sample	40 µl	-	-	-
Assay Buffer I	-	40 µl		
Reaction Buffer	50 µl	50 µl	-	-
Substrate	10 µl	10 µl	-	-
Mix well. Incubate at 37°C for 60 min				
Standard	-		100 µl	-
Distilled water	-		-	100 µl
Mix well. Read the OD at 405 nm.				

CALCULATION OF RESULTS

1. Unit Definition: One unit Caspase-9 activity is defined as the generates 1 μmol of pNA per hour in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 0.5 μmol/ml;

V_{Sample}: the volume of reaction sample, 40 μl = 0.04 ml;

$V_{Standard}$: the volume of reaction Standard, 100 μl = 0.1 ml;

V_{assay}: the volume of Assay Buffer I, 500 μl = 0.5 ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

T: the reaction time, 60 minutes.

B. Formula:

a). According to the protein concentration of sample

Caspase-9 activity (U/mg) =

$(OD_{Sample} - OD_{Control}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

$= 1.25 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

b). According to the weight of sample

Caspase-9 activity (U/mg) =

$(OD_{Sample} - OD_{Control}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$

$= 0.625 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the cell or bacteria

Caspase-9 activity ($U/10^4$) =

$(OD_{Sample} - OD_{Control}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay}) \times T]$

$= 0.625 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times N]$