Caspase 2 Assay Kit

ARG83406 Caspase 2 Assay Kit can be used to measure Caspase 2 in tissue extracts, cell lysate and other biological fluids.

Catalog number: ARG83406

Package: 96 wells

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INTRODUCTION

Caspase 2 also known as CASP2 is an enzyme that, in humans, is encoded by the CASP2 gene. CASP2 orthologs have been identified in nearly all mammals for which complete genome data are available. Unique orthologs are also present in birds, lizards, lissamphibians, and teleosts.

PRINCIPLE OF THE ASSAY

Caspase 2 Assay Kit determined Caspase 2 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 2 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 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.

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.

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 All 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 2 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-2 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-2 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-2 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-2 activity ($U/10^4$) =

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

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