Caspase 1 Activity Assay Kit

Caspase 1 Activity Assay Kit is a detection kit for the quantification of Caspase 1 activity in tissue extracts, cell lysates and other biological fluids.

Catalog number: ARG82810

Package: 96 wells

TABLE OF CONTENTS

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-1 / Interleukin-1 converting enzyme (ICE) is an evolutionarily conserved enzyme that proteolytically cleaves other proteins, such as the precursors of the inflammatory cytokines interleukin 1β and interleukin 18 as well as the pyroptosis inducer Gasdermin D, into active mature peptides. It plays a central role in cell immunity as an inflammatory response initiator. Once activated through formation of an inflammasome complex, it initiates a proinflammatory response through the cleavage and thus activation of the two inflammatory cytokines, interleukin 1β (IL-1β) and interleukin 18 (IL-18) as well as pyroptosis, a programmed lytic cell death pathway, through cleavage of Gasdermin D. The two inflammatory cytokines activated by Caspase-1 are excreted from the cell to further induce the inflammatory response in neighboring cells. [Provide by Wikipedia: Caspase 1]

PRINCIPLE OF THE ASSAY

This Caspase 1 Activity Assay Kit is a simple colorimetric assay that measures the amount of Caspase 1 activity in tissue extracts, cell lysates and other biological fluids. The assay is based on the enzyme driven reaction. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate. The pNA light emission can be quantified using a microtiter plate reader at 405nm. The concentration of Caspase 1 in the samples is then determined by comparing the O.D. 405 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Microplate	1 X 96-well plate	RT
Standard (500 µmol/L)	1 mL	4°C
Assay Buffer-A	2 x 30 mL (ready to use)	4°C
Assay Buffer-B	0.6 mL (ready to use)	4°C
Reaction Buffer	6 mL	4°C
Reducing Agent	1 vial (lyophilized)	-20°C
Substrate	1 vial (lyophilized)	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading in the 405 nm range
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is higher, please dilute the sample with PBS, or decrease the reaction time. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time to 2 hours, even overnight.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in 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.

Cell samples: Collect around 2 X 10^6 cells into centrifuge tube, centrifuged at 600 x g for 5 minutes at 4°C. Discard the supernatant after centrifugation, add 0.5 mL of Assay Buffer-A, 5 µL of Assay Buffer-B and 5 µL of Reducing Agent, mix and keep it on ice for 10 minutes. Centrifuged at 1,0000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection. Assay samples immediately or aliquot and store samples at ≤ -20 °C (store at -80°C is recommended) for up to one month. Avoid repeated freeze-thaw cycles.

Tissue samples: Weigh out 0.05 g tissue, homogenize with 0.5 mL Assay Buffer-A, 5 µL of Assay Buffer-B and 5 µL of Reducing Agent on ice for 10 minutes. Centrifuged at 1,0000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection. Assay samples immediately or aliquot and store samples at ≤ -20°C (store at -80°C is recommended) for up to one month. Avoid repeated freeze-thaw cycles.

Note: BCA method is not suitable for the determination of protein concentration. It is better to use Bradford method.

REAGENT PREPARATION

- **Reducing Agent:** Add **1 mL** of distilled water to dissolve before use. Mix thoroughly to make sure the reagent is dissolved completely before use. Aliquot and store the reconstituted Reducing Agent at -20°C.
- **Reaction Buffer working solution:** Add **0.1 mL** of Reducing Agent before use. Mix thoroughly before use.
- **Substrate:** Add **1 mL** of Reaction Buffer working solution to dissolve before use. Mix thoroughly and gently to make sure the reagent is dissolved completely before use and do not induce foaming. Aliquot and store the reconstituted Substrate at -20°C.

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate.

1. Add **40 µL** of **Samples** in Sample wells of the 96-well microplate.
2. Add **40 µL** of **Assay Buffer-A** in Control well of the 96-well microplate.
3. Add **50 µL** of **Reaction Buffer working solution** into Sample wells and Control well.
4. Add **10 µL** of **Substrate** into Sample wells and Control well.
5. Mix well and incubate plate for **1 hour** at **37°C** in the dark.
6. Add **100 µL** of **Standard** in another well as Standard well.
7. Add **100 µL** of **distilled water** in another well as Blank well.
8. Read the plate with a microplate reader at **405 nm**.

Note: If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time to 2 hours, even overnight.

Summary of Caspase 1 Activity Assay Procedure

Reagent	Sample	Control	Standard	Blank
Sample	40 µl	-	-	-
Assay buffer-A	-	40 µl	-	-
Reaction Buffer working solution	50 µl	50 µl	-	-
Substrate	10 µl	10 µl	-	-
Mix well and incubate plate for **1 hour** at **37°C** in the dark.				
Standard	-	-	100 µl	-
Distilled water	-	-	-	100 µl
Read the OD with a microplate reader at **405 nm** immediately.				

CALCULATION OF RESULTS

1. Unit Definition: One unit of Caspase-1 activity is defined as the enzyme generates 1 µmol pNA per hour.
2. Calculate the average absorbance value for each set of Standards, Blank, Control and samples.
3. Calculation:

A. Definition:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

N: the quantity of cell, N x 10^4;

$C_{Standard}$: the concentration of standard, 500 µmol/L = 0.5 µmol/mL;

$V_{Standard}$: the volume of standard, 0.1 mL;

V_{Sample}: the volume of sample, 0.04 mL;

V_{Assay}: the volume of Assay buffer, 0.5 mL;

T: the reaction time, 1 hour.

B. Formula:

a). According to the protein concentration of sample

Caspase 1 activity (U/mg)

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

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

b). According to the weight of sample

Caspase 1 activity (U/g)

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

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

c). According to the quantity of cells

Caspase 1 activity ($U/10^4$) =

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

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

4. Detection range:

   The detection range is from 5 µmol/L – 500 µmol/L.

5. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and this kit does not need serial diluted standards.