



Caspase-3 Microplate Assay Kit

Catalog # AS0166

Detection and Quantification of Caspase-3 Activity in Tissue extracts,
Cell lysate, Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

Contact information:

Tel:+1 (301) 446-2499 Fax:+1 (301) 446-2413

Email:techcn@signalwayantibody.com Web:www.sabbiotech.com

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
VI. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

I. INTRODUCTION

Caspases are members of the aspartate-specific cysteinyl protease family that play a central role in apoptosis. Apoptosis is involved in a variety of physiological and pathological events, ranging from normal fetal development to diseases such as cancer, organ failure, and neurodegenerative diseases.

Caspase-3 Microplate Assay Kit provides a convenient means to measure caspase-3 activity in biological samples. 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 colorimetric intensity is proportional to the caspase-3 activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30mlx 2	4 °C
Assay Buffer II	0.6 mlx 1	4 °C
Reaction Buffer	6 ml x 1	4 °C
Reducing Agent	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Standard (500µmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

Note:

Reducing Agent: add 1 ml distilled water to dissolve.

Reaction Buffer: add 0.1 ml Reducing Agent before use.

Substrate: add 1 ml Reaction Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, centrifuged at 600g 4°C for 5 minutes, discard the supernatant, add 0.5ml Assay Buffer I, 5µl Assay Buffer II and 5µl 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.

2. For tissue samples

Weigh out 0.05g tissue, homogenize with add 0.5ml Assay Buffer I, 5µl Assay Buffer II and 5µl 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.

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

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	40 μ l	--	--	--
Assay Buffer	--	40 μ l	--	--
Reaction Buffer	50 μ l	50 μ l	--	--
Substrate	10 μ l	10 μ l	--	--
Mix, put the plate into the oven, keep in dark, 37°C for 1 hour.				
Standard	--	--	100 μ l	--
Distilled water	--	--	--	100 μ l
Record absorbance measured at 405 nm.				

Note: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.

VI. CALCULATION

Unit Definition: One unit of Caspase-3 activity is defined as the enzyme generates 1 μmol pNA per hour.

1. According to the protein concentration of sample

$$\begin{aligned} \text{CASP3 (U/mg)} &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (\text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}) / \text{T} \\ &= 1.25 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{C}_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{CASP3 (U/g)} &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (\text{V}_{\text{Sample}} \times \text{W} / \text{V}_{\text{Assay}}) / \text{T} \\ &= 0.625 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{W} \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{CASP3 (U}/10^4) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (\text{V}_{\text{Sample}} \times \text{N} / \text{V}_{\text{Assay}}) / \text{T} \\ &= 0.625 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{N} \end{aligned}$$

$\text{C}_{\text{Standard}}$: the standard concentration, $500 \mu\text{mol/L} = 0.5 \mu\text{mol/ml}$;

$\text{V}_{\text{Standard}}$: the volume of standard, 0.1 ml;

$\text{C}_{\text{Protein}}$: the protein concentration of sample, mg/ml;

W: the weight of sample, g;

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

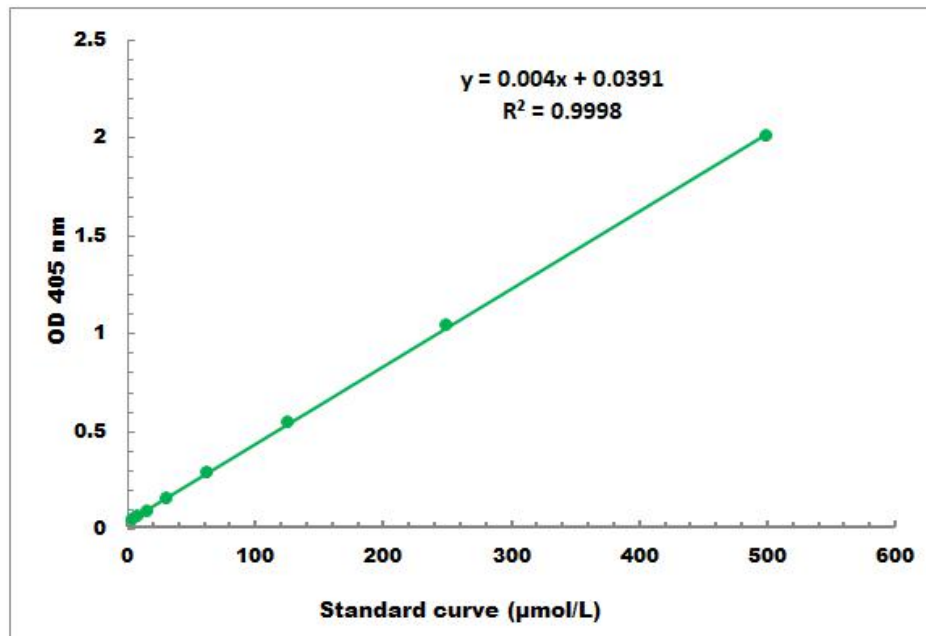
V_{Sample} : the volume of sample, 0.04 ml;

V_{Assay} : the volume of Assay Buffer, 0.5 ml;

T: the reaction time, 1 hour.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 5µmol/L -500µmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES