Caspase-9Microplate Assay Kit

User Manual

Catalog # CAK1217

(Version 1.4A)

Detection and Quantification of Caspase-9 (CASP9)activityin Tissue extracts, Cell lysate and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

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, andneurodegenerative diseases.

Caspase-9 Microplate Assay Kit provides a convenient means tomeasure caspase-9 activity in biological samples. The assay is basedon spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage fromthe labeled substrate. The pNA light emission can be quantified using a microtiter plate reader at 405nm. The colorimetricintensityis proportional to the caspase-9 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
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Note:

Reducing Agent: add 1 mldistilled waterto dissolve.

Reaction Buffer: add 0.1 ml Reducing Agent before use.

Substrate: add 1 ml Reaction Buffer todissolvebefore use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel 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 0.5mlAssay Buffer I, 5µl Assay Buffer II and 5µlReducing Agent, mix andkeep iton ice for 10 minutes. Centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.05g tissue, homogenize with0.5mlAssay Buffer I, 5µl Assay Buffer II and 5µlReducing Agent on ice for 10minutes.Centrifuged at 10000g 4°C for 10minutes, 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 intothe microplate:

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

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

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

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Caspase-9 activity is defined as the enzyme generates 1 μmolpNA per hour.

1. According to the protein concentration of sample

$$CASP9\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

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

2. According to the weight of sample

$$CASP9\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

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

3.According to the quantity of cells or bacteria

$$CASP9\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

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

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

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

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

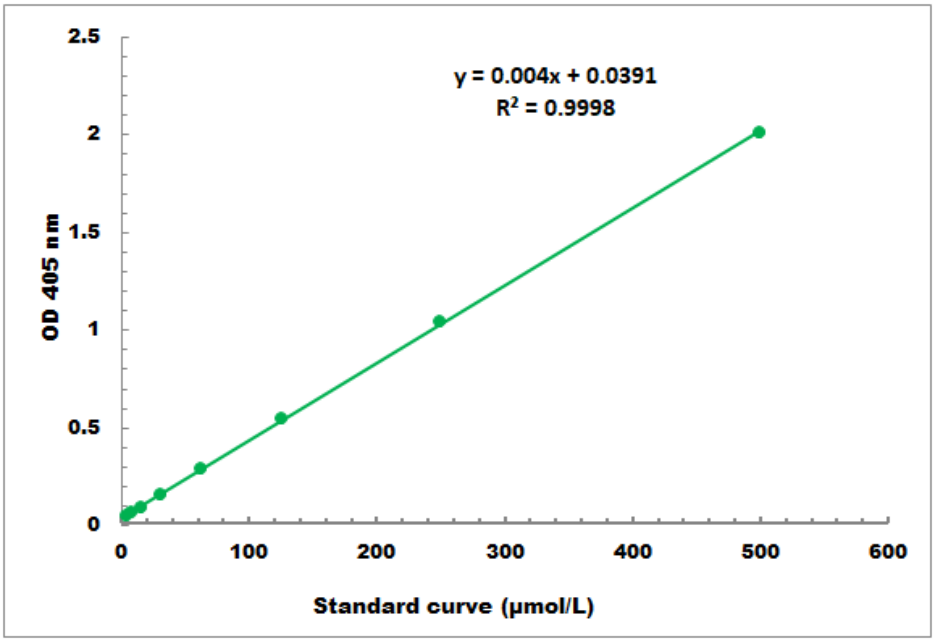
V_{Sample}: the volume of sample, 0.04 ml;

V_{Assay}: the volume of Assay Buffer I, 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 towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES