Caspase-5 Microplate Assay Kit

User Manual

Catalog # CAK1214

(Version 1.4A)

Detection and Quantification of Caspase-5 (CASP5) activity in Tissue extracts, Cell lysate and Other biological fluids Samples.

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, and neurodegenerative diseases.

Caspase-5 Microplate Assay Kit provides a convenient means to measure caspase-5 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-5 activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	0.6 ml x 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 (500 µmol/L)	1 ml x 1	4 °C
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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, 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 0.5 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.

2. For tissue samples

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.

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 µl	--	--	--
Assay Buffer I	--	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-5 activity is defined as the enzyme generates 1 μmol pNA per hour.

1. According to the protein concentration of sample

$$CASP5\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

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

2. According to the weight of sample

$$CASP5\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

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

3. According to the quantity of cells or bacteria

$$CASP5\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

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

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

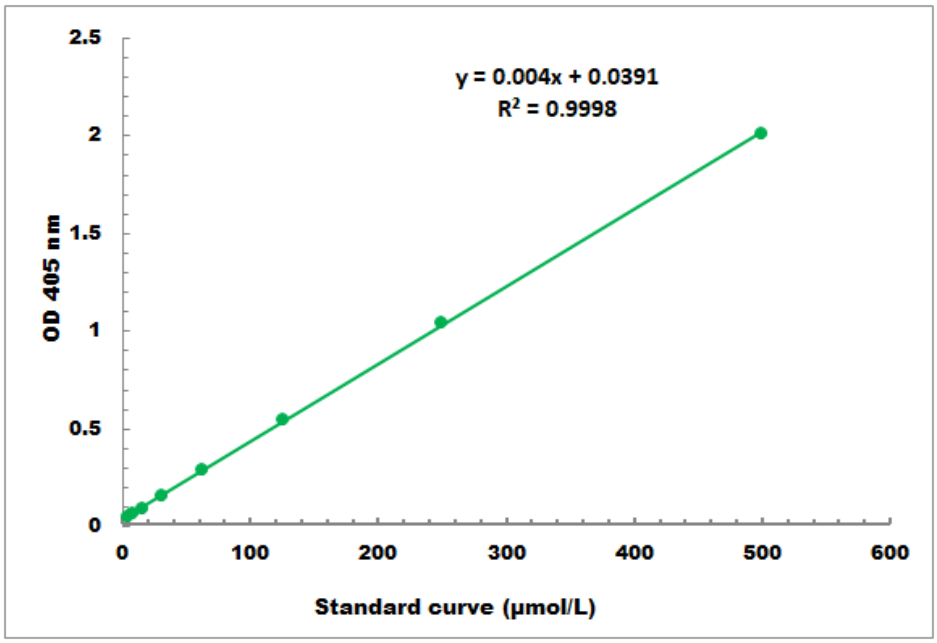
V_{Sample}: the volume of sample, 0.04 ml;

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: 5 μmol/L - 500 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES