

## **Caspase-9 Microplate Assay Kit**

**Cat #: orb707377 (manual)**

Detection and Quantification of Caspase-9 (CASP9) activity in Tissue extracts, Cell lysate and Other biological fluids Samples.

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

## 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-9 Microplate Assay Kit provides a convenient means to measure caspase-9 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-9 activity.

**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.

**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

## 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.

**ASSAY PROCEDURE**

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	40 $\mu$ l	--	--	--
Assay Buffer I	--	40 $\mu$ l	--	--
Reaction Buffer	50 $\mu$ l	50 $\mu$ l	--	--
Substrate	10 $\mu$ l	10 $\mu$ l	--	--
Mix, put the plate into the oven, keep in dark, 37 °C for 1 hour.				
Standard	--	--	100 $\mu$ l	--
Distilled water	--	--	--	100 $\mu$ l
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.

## CALCULATION

**Unit Definition:** One unit of Caspase-9 activity is defined as the enzyme generates 1  $\mu\text{mol}$  pNA per hour.

1. According to the protein concentration of sample

$$\begin{aligned} \text{CASP9 (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 1.25 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{CASP9 (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 0.625 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{CASP9 (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 0.625 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

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

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

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

W: the weight of sample, g;

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

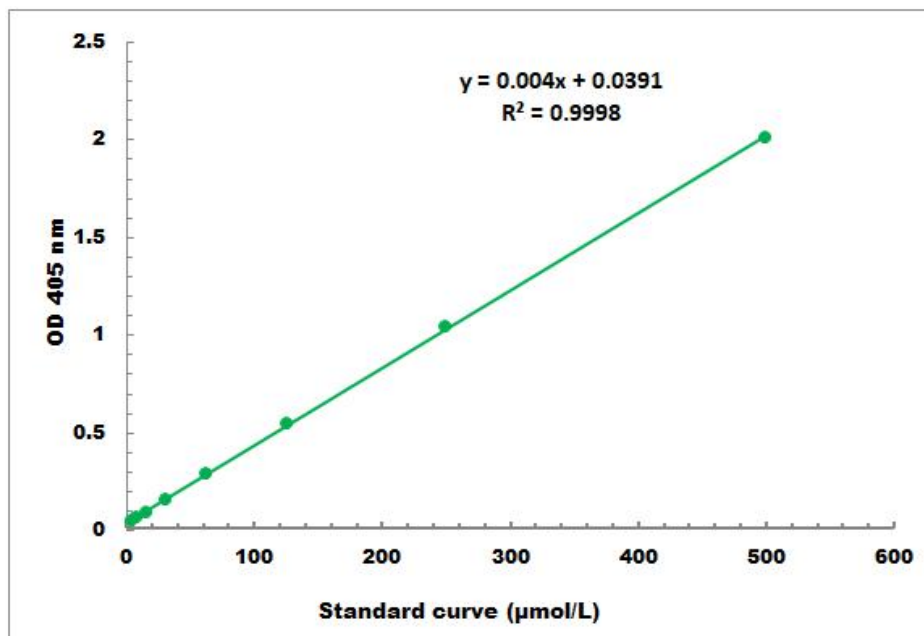
$V_{\text{Sample}}$ : the volume of sample, 0.04 ml;

$V_{\text{Assay}}$ : the volume of Assay Buffer I, 0.5 ml;

T: the reaction time, 1 hour.

## 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