# Caspase-2 Microplate Assay Kit

## Cat #: orb707371 (manual)

Detection and Quantification of Caspase-2 (CASP2) activity in Tissue extracts, Cell lysate and Other biological fluids Samples.

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

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## 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-2 Microplate Assay Kit provides a convenient means to measure caspase-2 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-2 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 |
| 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.

## 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

## 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 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.

## CALCULATION

**Unit Definition:** One unit of Caspase-2 activity is defined as the enzyme generates 1 μmol pNA per hour.

1. According to the protein concentration of sample

$$CASP2\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

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

2. According to the weight of sample

$$CASP2\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

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

3. According to the quantity of cells or bacteria of sample

$$CASP2\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

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

$C_{Standard}$: 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 × $10^4$;

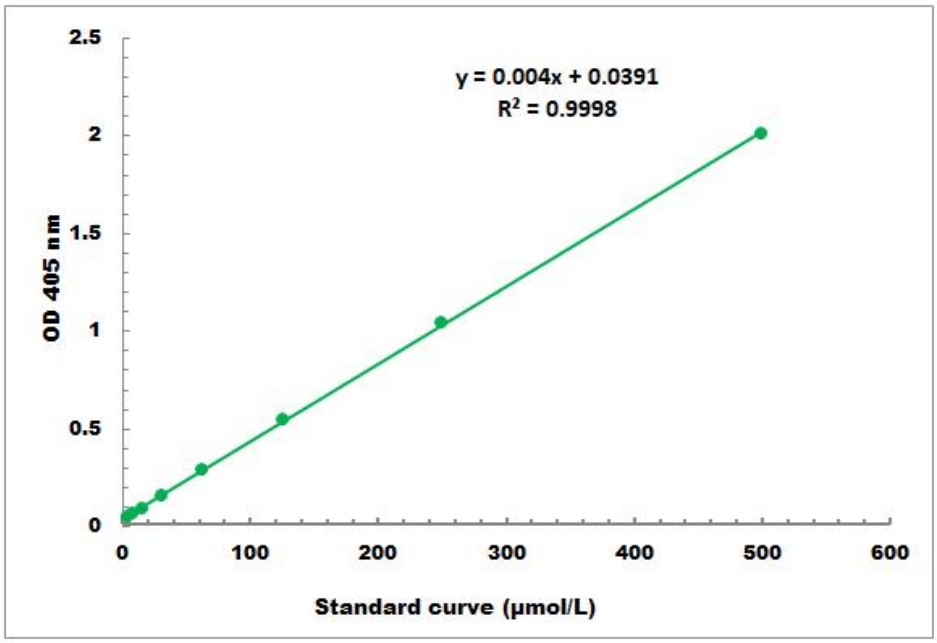
$V_{Sample}$: the volume of sample, 0.04 ml;

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

T: the reaction time, 1 hour.

## 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