Caspase-2 Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6802

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 .

1. 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 -2Activity Colorimetric Microplate Assay Kit provides a convenient means tomeasure caspase -2 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 -2 activity.

2. 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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3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8μmol/L.

Reducing Agent: Briefly centrifuge prior to opening. Add 1 mL distilled water to dissolve.Prepare for immediate use or store at -20°C for 3-4 weeks after reconstitution.

Reaction Buffer: Briefly centrifuge prior to opening. Add 0.1 mL Reducing Agent before use.

Substrate: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use. Keep in dark and store at 4°C for 1-2 weeks or -20°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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 I I and 5 μL Reducing Agent , mix and keep iton ice for 10 minutes. Centrifuged at 10000g 4°C for 1 0minutes, 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 0.5 mL Assay Buffer I , 5 μL Assay Buffer I I and 5 μL Reducing 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.

6. Assay Procedure

Add following reagents in to the microplate :

Reagent*	Sample**	Control	Standard	Blank
Sample	40 μl	--	--	--
Assay Bufferl	--	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 1-2 hours (or overnight).				
Standard	--	--	100 μl	--
Distilled water	--	--	--	100 μl
Record absorbance measured at 405 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

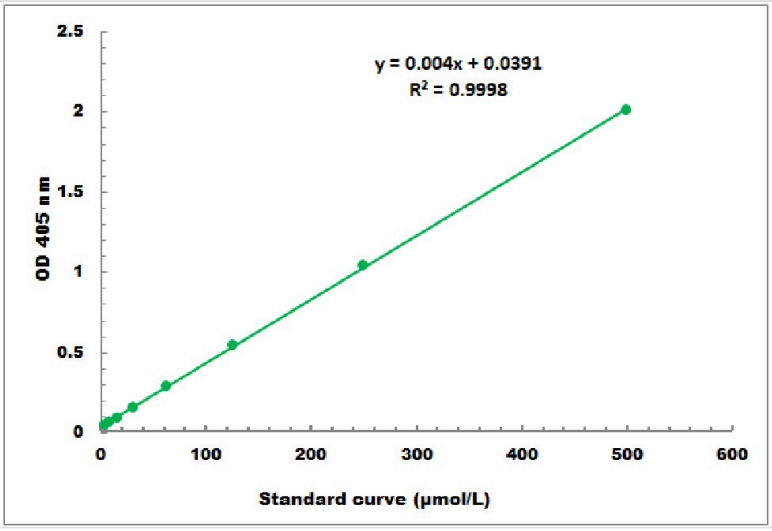
2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , hour

8. Typical Data

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



Detection Range : 5μmol/ L -500μmol/ L