Caspase-3 Assay
Cat. No. 8228, 100 tests

Introduction

Caspase-3 is a member of caspases that plays a key role in mediating apoptosis, or programmed cell death. Upon activation, it cleaves a variety of cellular proteins, causing morphological and functional changes to cells undergoing apoptosis. The ScienCell™ Caspase-3 Assay provides a quick and convenient method to measure caspase-3 activity. The colorimetric assay is based on the spectrophotometric detection of the chromophore p-nitroanilide (pNA) after its cleavage by caspases-3 from the labeled substrate acetyl-Asp-glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA). The concentration of pNA is measured by absorbance at 405 nm. The caspase-3 activity can be calculated as µmol of pNA released per minute per milliliter of cell lysate.

Kit Components

Cat. No.	# of vials	Reagent	Amount	Storage
8228a	1	Lysis Buffer	10 ml	2-8˚C
8228b	1	DTT Stock (1 M)	0.2 ml	-20˚C
8228c	1	10× Assay Buffer	3 ml	2-8˚C
8228d	1	Caspase-3 Substrate (2 mM)	1 ml	-20˚C, dark
8228e	1	pNA Standard (40 mM)	20 µl	-20˚C

Quality Control

ScienCell™ Caspase-3 Assay is applied to various concentration of active recombinant human caspase-3 (1.5 units, 5 units and 15 units per 100 µl reaction) with (2×10^{-3} µmol) and without caspase-3 inhibitor, according to Table 1. After incubation at 37˚C for 2 hours, absorbance at 405 nm is read. Results show that the OD_{405nm} increases as the concentration of caspase-3 increases, while 2×10^{-3} µmol of caspase-3 inhibitor inhibits the activity of up 15 units of caspase-3 effectively, which provides a negative control for the assay (Figure 1).

Procedures

A. Preparation of reagent

1. Aliquot and store DTT Stock (1 M) at -20˚C.
2. Add appropriate volume of 1 M DTT into the Lysis Buffer to a final concentration of 5 mM (dilute 200×) before each use. Lysis Buffer with 5 mM DTT is stable for less than a week at 2-8˚C.
3. Add appropriate volume of 1 M DTT into the 10× Assay Buffer to a final concentration of 50 mM (dilute 20×) before each use. 10× Assay Buffer with 50 mM DTT is stable for less than a week at 2-8˚C.

B. Preparation of pNA standard

1. Dilute 0.3 ml of 10× Assay Buffer with 50 mM DTT 10 times with DI H_2O to make 3 ml of working Assay Buffer.

2. Add 3 µl of 40 mM pNA stock to 297 µl of working Assay Buffer to make a 300 µl solution of 400 µM pNA.
3. Obtain 8 test tubes, add 300 µl of working Assay Buffer into each tube and label them #1 through #8.
4. Add 300 µl of the 400 µM pNA into tube #1 and mix well to get the 200 µM pNA standard.
5. Transfer 300 µl of 200 µM pNA standard from tube #1 to tube #2 and mix well to get the 100 µM pNA standard.
6. Repeat step 5 for tubes #3-7 to serially dilute the pNA standards. Do not add any pNA to tube #8, which serves as the blank.
7. Obtain a 96-well plate, add 100 µl/well of each pNA standard into the 96-well plate in triplicate to generate 0.02 µmol to 3.125×10^{-4} µmol/well standard, according to the following plate format:

	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**
A	0.02 µmol	0.01 µmol	5×10^{-3} µmol	2.5×10^{-3} µmol	1.25×10^{-3} µmol	6.25×10^{-4} µmol	3.125×10^{-4} µmol	Blank
B	0.02 µmol	0.01 µmol	5×10^{-3} µmol	2.5×10^{-3} µmol	1.25×10^{-3} µmol	6.25×10^{-4} µmol	3.125×10^{-4} µmol	Blank
C	0.02 µmol	0.01 µmol	5×10^{-3} µmol	2.5×10^{-3} µmol	1.25×10^{-3} µmol	6.25×10^{-4} µmol	3.125×10^{-4} µmol	Blank

8. Read samples at 405 nm on a microtiter plate reader. Plot the standard curve of OD_{405nm} vs. µmol of pNA (e.g. Figure 2). Determine the equation and R^2 value of the trend line.

C. Preparation of cell lysate

1. Induce apoptosis in cells by desired method.
2. Harvest cell pellet for each sample. Wash the cell pellet once with PBS. Count the number of cells.
3. Resuspend cells in pre chilled Lysis Buffer with 5 mM DTT at 1×10^7 cells/100µl; leave the cells on ice for 15 minutes with gentle agitation.
4. Centrifuge the lysed cells at 14,000 × g in pre-cooled centrifuge for 3 minutes, transfer the supernatant to a fresh tube and discard the pellet. Cell lysate can be stored at -70 ºC or used immediately for caspase-3 measurement.

D. Assay procedure

1. Sequentially add 20 µl of cell lysate, 10 µl of 10× Assay Buffer with 50 mM DTT, 60 µl of DI H_2O and 10 µl of 2 mM Caspase-3 Substrate to each well of a 96 well plate. Prepare a couple of blank wells by mixing 20 µl of Lysis Buffer with 5 mM DTT, 10 µl of 10× Assay Buffer with 50 mM DTT, 60 µl of DI H_2O and 10 µl of 2 mM Caspase-3 Substrate in each well of the 96 well plate. Incubate at 37˚C for 2-4 hours or until a yellowish color is developed. Record the time of reaction in minutes.
2. Read samples at 405 nm on a microtiter plate reader.

E. Calculation

1. Subtract the averaged OD_{405nm} of the blank wells from each of the sample well to get the calibrated OD_{405nm} values of the sample wells. Suppose the equation of the trend line of the pNA standard

curve is $y = Ax + B$, calculate the µmol of the pNA released in each sample well as follows:

$$pNA = \frac{OD_{405nm} - B}{A}$$

2. Calculate the caspase-3 activity in µmol pNA released per min per ml of cell lysate as follows:

$$Activity, \mu\text{mol pNA /min /mL} = \frac{\mu\text{mol of pNA}}{0.1mL\,(\text{lysate volume}) \times \text{t}}$$

Where t is the reaction time in minutes.

Table 1. Reaction scheme for capase-3 positive control with and without inhibitor.

	Caspase-3 1 unit/µl	**Caspase-3 Substrate 2 mM**	**Caspase-3 Inhibitor 0.2 mM**	**10× Assay Buffer with 50 mM DTT**	**DI H_2O**
Caspase-3 positive control without inhibitor	1.5 µl	10 µl	--	10 µl	78.5 µl
	5 µl	10 µl	--	10 µl	75 µl
	15 µl	10 µl	--	10 µl	65 µl
Caspase-3 positive control with inhibitor	1.5 µl	10 µl	10 µl	10 µl	68.5 µl
	5 µl	10 µl	10 µl	10 µl	65 µl
	15 µl	10 µl	10 µl	10 µl	55 µl

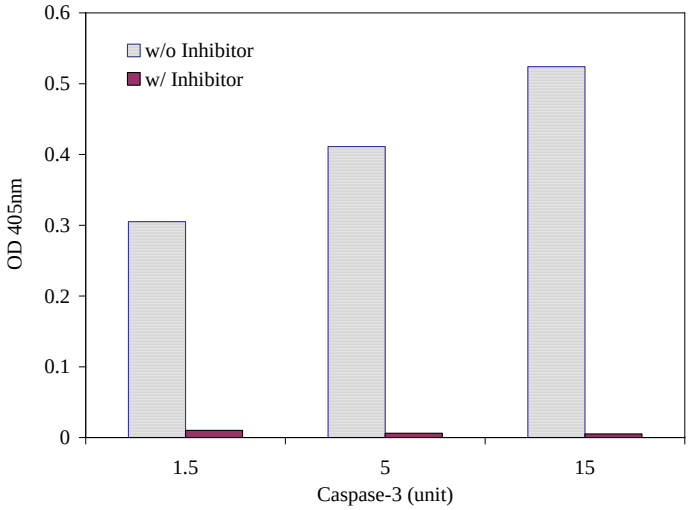

Figure 1. ScienCell™ Caspase-3 Assay kit applied to various amount of caspase-3 positive control with and without inhibitor (Table 1).

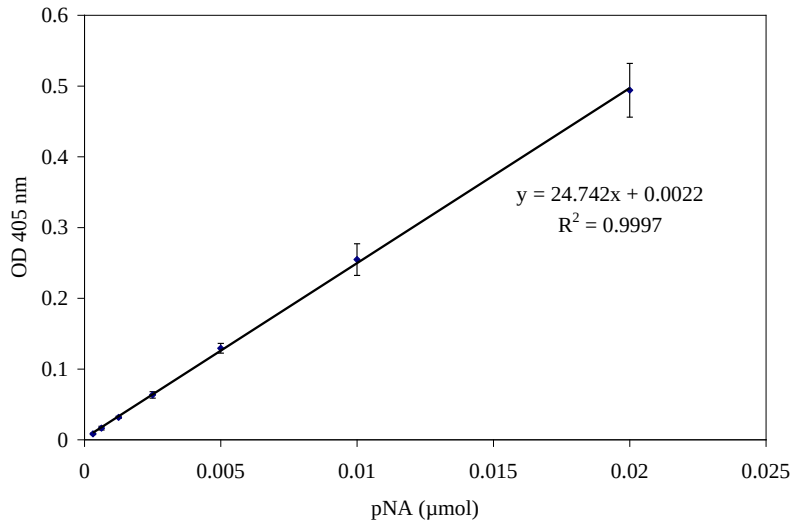

Figure 2. Standard curve of OD_{405nm} vs. pNA in µmol.