ScienCell™ Research Laboratories

Caspase-3 Assay Kit (CAS)

Catalog #8228
100 tests in 96-well plate

Product Description

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 (4 mM)	0.5 ml	-20˚C, dark
8228e	1	pNA Standard (40 mM)	20 µl	-20˚C

Materials supplied by user:

Colorimetric microplate reader
96-well plate

Quality Control

Caspase-3 activity was measured in primary cells treated with 1 µM Staurosporine for 6 hours using the ScienCell Caspase-3 Assay Kit (CAS). Absorbance at 405 nm after a 4-hour incubation at 37°C showed a concentration-dependent increase of caspase-3 activity, which was effectively reduced by a caspase-3 inhibitor.

Product Use

CAS is designed for detection of Caspase-3 activity in cells and tissues. This kit is for research use only and is not approved for human or animal use, or for application in clinical or in vitro diagnostic procedures.

Shipping

Dry Ice.

Storage

Upon receipt store the 8228a and 8228c at 4˚C. Store the 8228b, 8228d and 8228e at -20˚C.

Procedure

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 200 to 3.12 µM standards.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
pNA (µM)	200	100	50	25	12.5	6.25	3.12	Blank

8. Read standards at 405nm on a microplate reader.

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 5×10^6 cells/50µl; 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 -80 ºC or used immediately for caspase-3 measurement.
5. If enzyme activity per mg of enzyme is required, quantify the total protein with a protein quantification kit.

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 5 µl of 4 mM Caspase-3 Substrate to each well of a 96 well plate.
2. 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, 65 µl of DI H_2O and 5 µl of 4 mM Caspase-3 Substrate in each well of the 96 well plate.
3. Incubate at 37˚C for 2-4 hours or until a yellowish color is developed. If the color change is not obvious, the incubation time can be extended appropriately, or overnight. Record the time of reaction in hours. Read samples at 405 nm on a microplate reader.

E. Calculation

1. Average the OD_{405} nm of replicate wells of each pNA standard, test sample, control, and blank. Subtract the average OD_{405nm} value of the blank from the average OD_{405nm} values obtained with all other samples.
2. Based on the calibrated OD_{405nm} of the pNA standard, make a standard curve by plotting OD_{405nm} as a function of pNA concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. Assume the equation of the trend line of the standard curve is $y = Ax + B$, calculate the pNA concentration of test samples and controls as follows.

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

4. Fold-increase in Caspase-3 activity can be determined by comparing sample (treated) results with the level of the untreated control.

5. Multiply the concentration (in µM) by the volume of the reaction (in liters) to convert to nmol. Amount in nmol=$x_{sample} \times V_{reaction}$

   x_{sample} is the concentration in µM (which is µmol/L)

   $v_{reaction}$ is the volume of the reaction in liters. (1×10^{-4}, 100 µl/well)

6. Use the following formula to calculate the enzyme activity:

$$[Enzyme\ Activity\ (U/mL)] = \frac{nmol\ of\ pNA}{Reaction\ time\ (h) \times\ sample\ volume(mL)}$$

7. If you need to express the activity per mg of protein, divide by the protein concentration

$$[\text{Enzyme Activity (U/mg)}] = \frac{Enzyme\ Activity\ (U/mL)}{Protein\ Concnetration\ (mg/mL)}$$

Fig 1. Typical data of pNA standard curve.