Caspase 3 Assay Kit (Colorimetric)

Item NO.	Product Name
KTA3022	Caspase 3 Assay Kit (Colorimetric)

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201903

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INTRODUCTION

Background & Principle

Caspase family of cysteine proteases has been shown to play a key role in apoptosis. Mammalian caspases can be subdivided into three functional groups: initiator caspases (Caspase 2, 8, 9 and 10), executioner caspases (Caspase-3, 6 and 7), and inflammatory caspases (Caspase 1, 4, 5, 11 and 12). Caspase-3, also known as Yama, CPP32, and apopain, cleaves its substrates at the carboxyl terminus of aspartate residues. Active Caspase-3 consists of 2 sets of homodimers (17 and 12 kDa) that are derived from two precursor Caspase-3 polypeptides and has two active sites. Caspase-3, together with Caspases 8 and 9, is situated at pivotal junctions in apoptotic pathways.

Abbkine Caspase 3 Assay Kit (Colorimetric) is based on the hydrolysis of the peptide substrate Ac-DEVD-pNA (acetyl-Asp-Glu-Val-Asp p-nitroanilide) by caspase 3, resulting in the release of the p-nitroaniline (pNA), which can be quantified using a spectrophotometer or a microtiter plate reader at at 405 nm.

Storage/Stability

Stable for at least 12 months at recommended temperature from date of shipment. Gel pack with blue ice.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	20T	50T	100T	
Cell Lysis Buffer	5 mL	10 mL	20 mL	4°C
Reaction Buffer (2×)	5 mL	10 mL	20 mL	4°C,
Ac-DEVD-pNA (4 mM)	100 μL	250 μL	500 μL	-20°C, keep in dark
pNA (10 mM)	100 μL	250 μL	500 μL	-20°C, keep in dark
DTT (100×)	150 μL	400 μL	750 μL	-20°C

Other supplies required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Phosphate buffered saline (PBS)
- ELISA reader and flat-bottom, 96 well plates

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Reagent Preparation

Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay.

Cell Lysis Buffer: Add DTT to the Cell Lysis Buffer immediately before using (final concentration: add 10 µL of DTT (100×) stock per 1 mL of Cell Lysis Buffer). Keep on ice while using.

Reaction Buffer (1×): Dilute 2×Reaction Buffer 2-fold with water. Add DTT to the Reaction Buffer immediately before using (final concentration: add 10 µL of DTT (100×) stock per 1 mL of Reaction Buffer). Keep on ice while using.

pNA(10mM), Ac-DEVD-pNA (4mM) and DTT (100×): Store in aliquots at –20°C. Keep on ice while using.

Recommended procedures

A. Sample preparation

Note: We recommend using fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples at -80°C. Alternatively, if that is not possible, we suggest that you snap freeze cells in liquid nitrogen and store the samples immediately at -80°C. When ready to test your samples, thaw them on ice. Be aware his might affect the stability of your samples and the readings can be lower than expected.

Note： This kit detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.

1. Induce apoptosis in cells by desired method. Concurrently a control culture without induction is recommended for each Caspase 3 Colorimetric Assay.

2. a. For adherent cells, remove the culture medium and collect cells by trypsin digestion. pellet the cells by centrifugation at 600 × g for 5 minutes at 4 °C. Remove the supernatant by gentle aspiration. Wash the cell pellets twice with 1 mL of PBS. Centrifuge the cells and remove the supernatant completely by gentle aspiration. Resuspend 1-5 × 10^6 cells in 50 µL of chilled Cell Lysis Buffer

2. b. For suspension cells, pellet the cells by centrifugation at 500 × g for 5 minutes at 4 °C. Remove the supernatant by gentle aspiration. Wash the cell pellets twice with 1 mL of PBS. Centrifuge the cells and remove the supernatant completely by gentle aspiration. Resuspend 1-5 × 10^6 cells in 50 µL of chilled Cell Lysis Buffer.

2. c. For tissues, cut 5-20 mg of tissue into small pieces and place in a microcentrifuge tube. Wash tissue with PBS. Centrifuge tissue at 500 g for 5 minutes and discard the PBS.

Resuspend the tissue gently in 0.1 mL chilled Cell Lysis Buffer. Homogenize tissue with a Dounce homogenizer or a tissue grinder.

3. Incubate the lysate on ice for 15-20 minutes.

4. Centrifuge at 16,000 g for 15 minutes at 4 °C and transfer the supernatant to a new tube.

5. Assay protein concentration with Bradford method.

B. Caspase 3 activity Detection

1. pNA Calibration Curve Preparation: Prepare a series of pNA standard samples at the concentration range of 0 to 200 μM (0 μM, 10 μM, 20 μM, 50 μM, 100 μM, 200 μM) by diluting the pNA stock solution with 1×Reaction Buffer (containing DTT).

2. Add 50 μL of 1×Reaction Buffer (containing DTT) to each 50 μL sample (samples, background control and standard samples).

3. Add 5 μL of the 4 mM Ac-DEVD-pNA substrate (200 μM final concentration). Mix well and incubate at 37°C for 1-2 hour.

4. Read samples at 405nm in a microtiter plate reader, or spectrophotometer.

Note: Background reading from cell lysates and buffers should be subtracted from the readings of both induced and the uninduced samples before calculating fold increase.

CALCULATIONS

Note: Background reading from cell lysates and buffers should be subtracted from the readings of both induced and the uninduced samples before calculating fold increase.

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

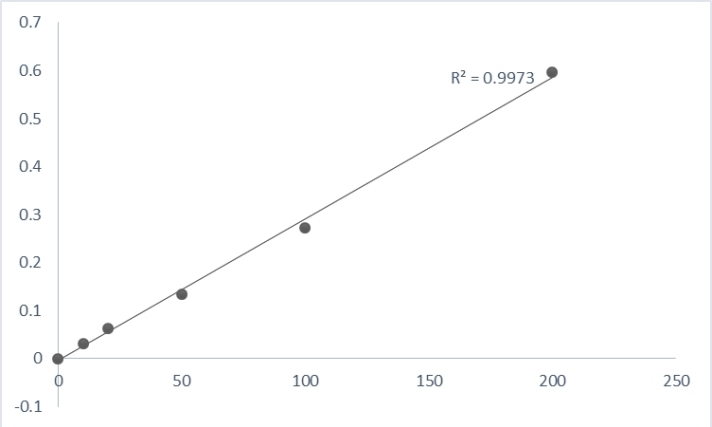

Fig1. Typical data of pNA standard curve

Calculate the caspase 3 activity in μmol pNA released per min per mL of cell lysate.

Activity, μmol pNA/min/mL = μmol pNA /(t x v)

v = volume of sample in mL
t =reaction time in minutes