Product Manual

Caspase 3 Assay Kit

Catalog No.: abx294003

Size: 20 tests / 50 tests / 100 tests

Storage: Store the DTT and Ac-DEVD-pNA reagents at -20°C. The Ac-DEVD-pNA reagent should be stored in the dark. Store the Lysis Buffer and Reaction Buffer at 4°C for short-term storage, otherwise store at -20°C for long-term storage. Avoid repeated freeze/thaw cycles. Before use, warm all reagents to room temperature.

Introduction

Caspases (Cysteine-requiring Aspartate protease) are a family of proteases that mediate cell death and are important to the process of apoptosis. Caspase 3 (also referred to as CPP32, Yama, and apopain) is a member of the CED3 subfamily of caspases and is one of the critical enzymes of apoptosis. Caspase 3, which is an effector caspase, is the most studied of the mammalian caspases. It can process procaspase 2, 6, 7, and 9 and specifically cleave most of caspase-related substrates known to date, including many key proteins such as the nuclear enzyme poly(ADP-ribose) polymerase (PARP), the inhibitor of caspase-activated deoxyribonuclease (ICAD), and gelsolin and fodrin, which are proteins involved in apoptosis regulation. This cleavage is part of the mechanism leading to cell death. In addition, caspase 3 plays a central role in mediating nuclear apoptosis including chromatin condensation and DNA fragmentation.

Principle of the assay

This kit is based on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) by Caspase 3, resulting in the release of the p-nitroaniline (pNA) moiety. pNA has a high absorbance at 405 nm. The concentration of pNA released from the substrate is calculated from the absorbance value at 405 nm.

Kit components (20 tests)

1. Lysis buffer: 5 ml
2. Reaction buffer: 1.0 ml
3. Ac-DEVD-pNA: 100 µl
4. DTT: 50 µl

Kit components (50 tests)

1. Lysis buffer: 10 ml
2. Reaction buffer: 2.5 ml
3. Ac-DEVD-pNA: 250 µl
4. DTT: 100 µl

Kit components (100 tests)

1. Lysis buffer: 15 ml
2. Reaction buffer: 5.0 ml
3. Ac-DEVD-pNA: 500 µl
4. DTT: 150 µl

Materials required but not provided

1. 37°C incubator
2. Spectrophotometer or plate reader (wavelength 405 nm)
3. Centrifuge and centrifuge tubes
4. Precision pipette and disposable pipette tips
5. Phosphate-buffered saline (PBS)
6. Reagents or kit for total protein detection
7. Glass pestle (if using tissue samples)

A. Reagent Preparation

Before carrying out the assay, place all reagents and samples in an ice bath.

1. In a separate tube, add 0.5 µl of DTT to each 50 µl of Lysis Buffer to make up the Working Lysis Buffer. Place on ice before use.
2. Mix the Reaction Buffer and Ac-DEVD-pNA well and place on ice before use.

B. Sample Collection

1. Prepare cell or tissue samples as below:

- **Adherent cells:** Adherent cells should be detached with trypsin and then collected by centrifugation. Centrifuge for 5 minutes at 2000 RPM, then discard the supernatant. Re-suspend the adherent cells in 1 ml cold PBS, centrifuge for 5 minutes at 2000 RPM, then discard the supernatant. Re-suspend the cells in Working Lysis Buffer (add 50 µl of Working Lysis Buffer to 2×10^6 cultured cells), then place in an ice bath for 30 mins. Vortex the mixture 3-4 times whilst in the ice bath.
- **Suspension cells:** Suspension cells can be collected from the sediment by centrifugation directly. Re-suspend the cells in 1 ml cold PBS, centrifuge for 5 minutes at 2000 RPM, then discard the supernatant. Re-suspend the cells in Working Lysis Buffer (add 50 µl of Working Lysis Buffer to 2×10^6 cultured cells), then place in an ice bath for 30 mins. Vortex the mixture 3-4 times whilst in the ice bath.
- **Tissue:** Add 50 µl of cold Working Lysis Buffer to 50 mg of ground tissue. Transfer the tissue homogenate to a new tube, then place in an ice bath for 5 mins.

2. Centrifuge at 12,000 RPM and 4°C for 10-15 min.
3. Pipette the supernatant into a new tube and place on ice. Assay immediately or store at -70°C.

C. Assay Procedure

1. Determine the total protein in the samples using a quantitative total protein assay kit.
2. In separate tubes, prepare the blank and samples according to the table below. 50 µl of sample should contain between 100 – 200 µg of protein. If the sample volume is less than 50 µl, add Working Lysis Buffer to make up 50 µl. Add the Ac-DEVD-pNA reagent last.

	Blank	Sample
Reaction buffer (µl)	50	50
Working Lysis Buffer (µl)	50	-
Sample (µl)	-	50
Ac-DEVD-pNA (µl)	5	5
Total volume (µl)	105	105

3. Ensure that the solution in each tube is mixed thoroughly. Avoid bubbles and foaming. Incubate for 4 hours at 37°C. The reaction time can be extended if required, including overnight.
4. Determine the absorbance at 405 nm (or 400 nm) with a spectrophotometer or a microplate reader.
5. The activity of Caspase 3 can be calculated by: OD_{Sample} / $OD_{Negative\ Control}$.