Caspase-3/CPP32 Colorimetric Protease Assay

Catalog# KHZ0021 (25 Tests)
KHZ0022 (200 Tests)

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INTENDED USE

The Apo*Target*™ Caspase-3 Protease Assay is to be used for the *in vitro* determination of Caspase-3 proteolytic activity in lysates of mammalian cells. This test is designed for research use only. It is not to be used in diagnostic procedures.

PRINCIPLE OF THE METHOD

Caspase-3 (also known as CPP32, Yama or apopain) is a member of the Interleukin-1β Converting Enzyme (ICE) family of cysteine proteases. Caspase-3 exists in cells as an inactive 32 kDa proenzyme, called pro-Caspase-3. Pro-Caspase-3 is cleaved into active 17 and 12 kDa subunits by upstream proteases such as Caspase-6 (Mch2), Caspase-8 (FLICE) and Granzyme B during apoptosis. The downstream substrates of Caspase-3 include poly (ADP Ribose) Polymerase (PARP), Sterol Regulatory Element Binding Proteins (SREBPs), nuclear lamins and others. The overexpression of Caspase-3 can result in apoptosis. Likewise, the inhibition of Caspase-3 or other caspases can prevent cells from entering the apoptotic pathway.

The Caspase-3 colorimetric protease assay provides a simple and convenient means for quantitating caspases that recognize the amino acid sequence, DEVD. The kit includes substrate and optimized buffers. The substrate, DEVD-*p*NA, is composed of the chromophore, *p*-nitroanilide (*p*NA), and a synthetic tetrapeptide, DEVD (Asp-Glue-Val-Asp), which is the upstream amino acid sequence of the Caspase-3 cleavage site in PARP. Upon cleavage of the substrate by Caspase-3 or related caspases, free *p*NA light absorbance can be quantified using a spectrophotometer or a microplate reader at 400 or 405 nm. Comparison of the absorbance of *p*NA from apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-3 activity.

REAGENTS PROVIDED

Note: Store kit at -20°C. Once opened, store Cell Lysis Buffer, 2x Reaction Buffer, and Dilution Buffer at 2-8°C. Refer to reagent label for experation.

1. Cell Lysis Buffer (25 mL or 100 mL): tris buffered saline containing detergent.
2. 2x Reaction Buffer (2 mL or 16 mL): contains buffered saline, glycerol and detergent.
3. Substrate (125 µL or 1000 µL): contains 4 mM of synthetic peptide (sequence: DEVD) conjugated to the chromophore, *p*NA, in DMSO.
4. Dilution Buffer (25 mL or 200 mL): buffered solution containing HEPES, Tris and NaCl.
5. DTT (100 µL or 800 µL): contains 1 M dithiothreitol.

MATERIALS NOT PROVIDED

1. Spectrophotometer (and cuvettes) or microplate reader capable of measurement at 400-405 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips.
3. Protein measurement method such as Bradford protein assay.
4. Tubes appropriate for holding cells during induction of apoptosis.
5. Microcentrifuge.
6. Reaction tubes or 96-well microplate.

PROCEDURAL NOTES/ LAB QUALITY CONTROL

1. When not in use, kit components should be stored refrigerated or frozen as indicated on vial or bottle labels. All reagents should be warmed to room temperature before use.
2. If not analyzed immediately, samples should be stored at -20ºC or lower.
3. It is recommended that all samples and controls be run in duplicate.
4. Protect DEVD-*p*NA from light.
5. Cover or cap all reagents when not in use.
6. Do not mix or interchange different reagent lots from various kits.

7. Do not use reagents beyond the expiration date of the kit.
8. Set negative controls using same number of cells or same amount of lysates without adding DEVD-*p*NA substrate.

WARNINGS AND PRECAUTIONS

1. This kit is intended for research use only. It is not to be used for diagnostic procedures.
2. Never pipette by mouth.
3. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

ASSAY PROCEDURE

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet 3-5 x 10^6 cells per sample.
3. Resuspend cells in 50 µL of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 minute in a microcentrifuge (10,000 x g).

Note: This step is not recommended if using the fluorometric caspase assay.

5. Transfer supernatant (cytosol extract) to a fresh tube and put on ice.
6. Assay protein concentration by any standard method.
7. Dilute each cytosol extract to a concentration of 50-200 µg protein per 50 µL Cell Lysis Buffer (1-4 mg/mL).
8. Determine the number of samples to be measured and aliquot enough 2x Reaction Buffer into a glass tube (assuming 50 µL of 2x Reaction Buffer per sample). Add DTT to the 2x Reaction Buffer immediately before use (10 mM final concentration: add 10 µL of 1.0 M DTT stock per 1 mL of 2x Reaction Buffer).
9. Add 50 µL of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
10. Add 5 µL of the 4 mM DEVD-*p*NA substrate (200 µM final concentration) and incubate at 37°C for 2 hours**.** Keep the samples in the dark during incubation.
11. Read samples at 400 nm or 405 nm in a microplate reader, or spectrophotometer using a 100 µL micro-quartz cuvette, or dilute sample to 1 mL with Dilution Buffer and use a regular cuvette.

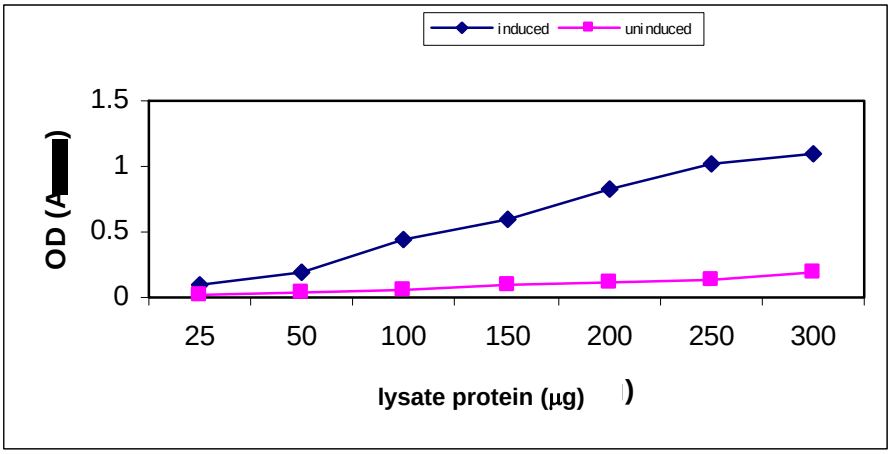
Note: Dilution of the samples proportionally decreases the optical density. You may also perform the entire assay directly in a 96-well plate.

12. Fold-increase in Caspase-3 activity should be determined by direct comparison to the level of the uninduced control.

Note: Background readings from cell lysates and buffers should be subtracted from the readings of both induced and the uninduced samples before calculating fold-increase in Caspase-3 activity.

TYPICAL DATA

The following absorbance data were obtained from Jurkat cells. Apoptosis was induced by incubating Jurkat cells with 0.2 µg/mL of anti-Fas monoclonal antibody for 8 hours. The assay was done according to the procedure described above. Typically, the Caspase-3 activity in 20 µg-200 µg protein per sample can be detected.



LIMITATIONS OF THE PROCEDURE

This kit provides a simple and convenient method to detect Caspase-3 activity of apoptotic cells. A relatively high concentration of DTT (10 mM) is required for full activity of the recombinant enzymes. Make sure that DTT is added to Reaction Buffer when the assay is carried out. Otherwise, unexpected low Caspase-3 activity will occur. Turbidity, lipemia or particulate materials in samples can decrease the assay precision.

REFERENCES:

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