



**Caspase Colorimetric Protease Assay
Sampler Kit
(Caspases-2, -3, -6, -8, -9)
Catalog# KHZ1001 (125 Tests)**



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INTENDED USE

The *ApoTarget*[™] Caspase Colorimetric Protease Assay is to be used for the *in vitro* quantitative determination of caspase 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

Apoptosis, or programmed cell death, is the outcome of a programmed intracellular cascade of genetically determined steps. One of the mechanisms which is consistently implicated in apoptosis is the activation of a group of intracellular cysteine proteases called caspases. Caspases play a critical role in the execution phase of apoptosis and are responsible for many of the biochemical and morphological changes associated with apoptosis.

Caspases share similarities in amino acid sequence, structure, and substrate specificity. Thus far, 14 caspases have been identified. Caspases have been grouped according to sequence homology into three subclasses: the caspase-1 subfamily (caspases-1, -4, -5, -11, -12, and -13); the caspase-3 subfamily (caspases-3, -6, -7, -8, and -10); the caspase-2 subfamily (caspases -2 and -9). Functionally, some caspases (such as caspases-2, -8 and -9) act as initiator or signal caspases, while others (such as caspases-3, -6 and -7) act as effectors of apoptosis. Some caspases, notably those resembling caspase-1, play important roles in inflammation by activating cytokines.

Caspases are present as inactive pro-enzymes that are activated by proteolytic cleavage. The pro-enzymes are proteins of 30-50 kDa that contain three domains: an NH₂-terminal domain, a large subunit (~20 kDa) and a small subunit (~10 kDa). Activation involves proteolytic processing between domains, followed by association of the large and small subunits to form heterodimers, the active form of the caspases. Activated caspases can cleave procaspases in the caspase cascades and many intracellular proteins including lamins, PARP (poly ADP-ribose polymerase), and DFF45/ICAD, etc. Cleavage of DFF45/ICAD is a critical step leading to the internucleosomal cleavage of DNA (DNA fragmentation), a characteristic hallmark of apoptosis.

The caspase colorimetric protease assay sampler kit provides a simple and convenient means for quantitating the enzyme activity of caspases that recognize the amino acid sequence, VDVAD (for caspase-2), DEVD (for caspase-3), VEID (for caspase-6), IETD (for caspase-8), and LEHD (for caspase-9). The substrates provided for measuring the activity of these caspases are synthetic peptides which are labeled at their C-termini with para-nitroaniline (*p*NA). Upon cleavage of the substrates by caspases, absorption of light by free *p*NA can be quantified using a spectrophotometer or a microtiter plate reader at 400 or 405 nm. Comparison of the absorbance of *p*NA from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase activity.

REAGENTS PROVIDED

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

1. Cell Lysis Buffer (100 mL): Tris buffered saline containing detergent.
2. 2x Reaction Buffer (16 mL): contains buffered saline, glycerol and detergent.
3. Substrates contain 4 mM of synthetic peptides conjugated to the chromophore, *p*NA (*p*-nitroanilide), in DMSO.
VDVAD-*p*NA (Caspase-2): 125 µL
DEVD-*p*NA (Caspase-3): 125 µL
VEID-*p*NA (Caspase-6): 125 µL
IETD-*p*NA (Caspase-8): 125 µL
LEHD-*p*NA (Caspase-9): 125 µL
4. DTT (800 µL): contains 1 M dithiothreitol.

MATERIALS NOT PROVIDED

1. 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. 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.
2. If not analyzed immediately, samples should be stored at -20°C or lower.
3. It is recommended that all samples and controls be run in duplicate.
4. Protect substrates 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 the same number of cells or same amount of lysates without adding 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.

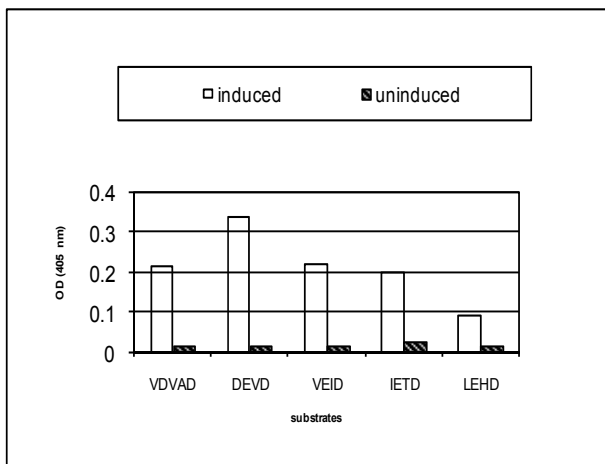
ASSAY PROCEDURE

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet $3-5 \times 10^6$ cells per sample.
3. Resuspend cells in 50 μ L 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).
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 μ g protein per 50 μ L 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 μ L of 2x Reaction Buffer per sample). Add DTT to the 2x Reaction Buffer immediately before use (10 mM final concentration: add 10 μ L of 1.0 M DTT stock per 1 mL of 2x Reaction Buffer).
9. Add 50 μ L of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
10. Add 5 μ L of the 4 mM substrate (200 μ M final concentration) and incubate at 37°C for 1-2 hours. Keep the samples in the dark during incubation.
11. Read sample in a 400 nm or 405 nm microplate reader.
12. Fold-increase in caspases-2, -3, -6, -8, and -9 activity should be determined by direct comparison to the level of the uninduced control.

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

TYPICAL DATA

The following absorbance data were obtained from Jurkat cells. Apoptosis was induced by incubating Jurkat cells with 10 µg/mL camptothecin for 6 hours. The assay was performed according to the procedures described above.



LIMITATIONS OF THE PROCEDURE

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

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