Caspase-2 Colorimetric Assay

Catalog Number: BF8100
100 Tests

Reagents Provided

VDVAD-pNA Substrate: 500 µL of 4 mM VDVAD substrate peptide conjugated to p-nitroaniline.

Lysis Buffer: 100 mL of Lysis Buffer (narrow-mouth bottle)

Reaction Buffer 2: 4 x 2.0 mL vials of 2X Reaction Buffer 2

DTT: 400 µL of a 1 M solution of dithiothreitol

Dilution Buffer: 100 mL of Dilution Buffer (wide-mouth bottle)

Upon arrival, store the entire kit at ≤ -20° C in a manual defrost freezer. After the first use, only the Substrate and the DTT solution need to be returned to ≤ -20° C storage. The remaining reagents (buffers) may be stored at 2 - 8° C. Avoid repeated freeze-thaw cycles.

References

1. Kerr, J.F.R. *et al.* (1972) Br. J. Cancer. **26**:239.
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3. Thompson, C.B. (1995) Science **267**:1456.
4. Vermes, I. *et al.* (1995) J. Immunol. Meth. **184**:39.
5. Darzynkiewicz, Z. *et al.* (1992) Cytometry **13**:795.
6. Wang, L. *et al.* (1994) Cell **78**:739.
7. Cryns, V. and Yuan, J. (1998) Genes Develop. **12:**1551.
8. Talanian, R.V. et al. (1997) J. Biol. Chem. **272**:9677.

Intended Use

To determine the increased enzymatic activity of the caspase-2 class of proteases in apoptotic cells by colorimetric reaction.

Background Information

Apoptosis was originally described as a mechanism of controlled or physiological cell death (1). It is associated with the regulation of cellular homeostasis in organs and the elimination of damaged cells or of cells with deleterious reactivities from the host. Apoptosis is very common in tissues with intense hematopoietic activity (*e.g.* bone marrow and thymus) and in organs with high proliferative activity. Additionally, apoptosis has been implicated in the progression of a number of pathological conditions, including AIDS, cancer and autoimmune diseases (2, 3).

Apoptosis is characterized by a variety of cellular changes including loss of membrane phospholipid asymmetry (4), chromatin condensation, mitochondrial swelling and DNA cleavage (5). The end result of these changes is a form of cell death that avoids the normal inflammatory response associated with necrosis.

Caspase-2, also known as Ich-1 and Nedd-2 (6), is an intracellular cysteine protease that exists as a proenzyme, becoming activated during the cascade of events associated with apoptosis. Caspase-2 mRNA can be spliced into two isoforms: the long form is pro-apoptotic while the short form is anti-apoptotic (7). Caspase-2 message can be found in variety of cells of different lineages including THP-1, U937, Jurkat and HeLa cells (6). The presence of caspase-2 in cells of different lineages suggests that caspase-2 is a key enzyme required for the execution of apoptosis (8). The preferred cleavage substrate for caspase-2 is VDVAD (8).

Principle of the Test

Cells that are suspected or have been induced to undergo apoptosis are first lysed to collect their intracellular contents. The cell lysate can then be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule p-nitroaniline (pNA). The cleavage of the peptide by the caspase releases the chromophore pNA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

Sample Preparation

1. Cells that have been induced to undergo apoptosis are collected by centrifugation in a conical tube at 250 x g for 10 minutes (**Note:** *we recommend counting the cells before pelleting them*). The supernate is gently removed and discarded while the cell pellet is lysed by the addition of the Lysis Buffer. The amount of Lysis Buffer to be added to the pellet is determined by the number of cells present (this can be estimated from the number of cells initially cultured). Add 25 μL of cold Lysis Buffer per 1 x 10^6 cells.
2. The cell lysate is incubated on ice for 10 minutes and then centrifuged at 10,000 x g for 1 minute. Transfer the supernate to a new tube and keep on ice. This should yield a cell lysate with an approximate protein concentration of 2 - 4 mg/mL. The protein content of the cell lysate can be estimated using a protein determination assay that is compatible with detergents present in the Lysis Buffer (*e.g.* BCA Protein Assay, Pierce Chemical Co., Catalog # 23225).
3. The enzymatic reaction for caspase activity is best carried out in a 96 well flat bottom microplate that can be read with a microplate reader.
4. Each reaction requires 50 μL of cell lysate (*i.e.* derived from 2 x 10^6 or 100 - 200 μg of total protein). If larger volumes of cell lysate are necessary to meet the above requirements, the total reaction volume may be scaled up (*i.e.* the volume of <u>each</u> reagent added should be proportionally increased).
5. Each reaction also requires 50 μL of 2X Reaction Buffer 2. Prior to using the 2X Reaction Buffer 2, add 10 μL of fresh DTT stock per 1 mL of 2X Reaction Buffer 2.
6. To each reaction well add 5 μL of caspase-2 colorimetric substrate (VDVAD-pNA).
7. Incubate the plate at 37°C for 1 - 2 hours.
8. Read the plate on a microplate reader using 405 nm wavelength light. If readings are not in the linear range of the instrument, repeat the assay by diluting the cell lysates with the Dilution Buffer.
9. Additional controls that should be included in this assay are a) no cell lysate and b) no substrate. **A recombinant caspase-2 enzyme is available for use as a positive control (R&D Systems' Catalog # 702-C2) or standard.** The total reaction volume must be kept constant and therefore distilled water can be used to replace the volume normally occupied by either the cell lysate or the substrate reagent.
10. For comparative analysis, the above assay should be repeated with non-induced cells.

This protocol may require modification, depending upon final utilization.

The results are best expressed as fold increase in caspase activity of apoptotic cells over that of non-induced cells. If the background controls (reactions where no cell lysate is added or where no VDVAD-p-NA substrate is added) give a substantial reading, it is recommended that these values be subtracted from the experimental results prior to calculating the fold increase.