



CELL TECHNOLOGY, INC.

APO 3 HTS™

High Throughput Assay for Caspase 3/7

Key Benefits

- Homogenous assay for active caspase 3/7.
- Breakthrough in cell lysis buffer and preservation of caspase activity
Results in a **No wash** one-step assay.
- No need to wash out media from cell samples, just add the reagent directly to your experimental samples.
- Easy to Use: No need to make cell lysates or run western blots.
- Works with suspension and adherent cells.

Background

Apoptosis is an evolutionarily conserved form of cell suicide, which follows a specialized cellular process. The central component of this process is a cascade of proteolytic enzymes called caspases. These enzymes participate in a series of reactions that are triggered in response to pro-apoptotic signals and result in cleavage of protein substrates, causing the disassembly of the cell (1).

Caspases have been identified in organisms ranging from *C. elegans* to humans. The mammalian caspases play distinct roles in apoptosis and inflammation. In apoptosis, caspases are responsible for proteolytic cleavages that lead to cell disassembly (effector caspases), and are involved in upstream regulatory events (initiator caspases). An active caspase consists of two large (~20 kD) and two small (~10 kD) subunits to form two heterodimers which associate in a tetramer (2-4). As is common with other proteases, caspases are synthesized as precursors that undergo proteolytic maturation, either autocatalytically or in a cascade by enzymes with similar specificity (5). Caspase 3, also known as CPP-32, Apopain or Yama, is a key effector caspase in the apoptotic pathway (6). It is present in many different cell lineages and is responsible for the cleavage of a variety of molecules such as poly ADP-ribose polymerase (PARP), protein kinase Cd, actin and DNA-dependent protein kinase (7,8).

Assay Principle

Cell Technology's Apo 3/7 HTS™ Assay utilizes the quenched (z-DEVD)₂-R110 peptide substrate for caspase 3/7 detection. The absorption and emission properties of the R110 dye are suppressed when attached to the z-DEVD peptide sequence. When R110 is cleaved away, by active caspase3/7, from the quenching DEVD sequence, the free dye excites at 488nm and emits at 515-530 nm.

As a result of a novel and proprietary Lysis Buffer System, the Apo 3/7 HTS™ Assay is a homogenous platform that can be utilized for high throughput fluorescence plate reader applications. The reagent is directly added to the samples thus eliminating any wash steps.

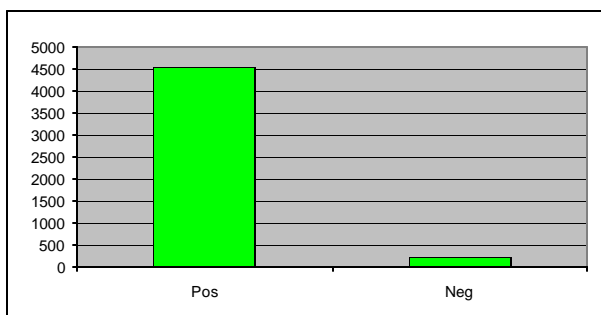


Figure 1. U937 cells were stimulated with 1µM staurosporine for 5 hours, after which, caspase 3/7 activity was analyzed using the Apo 3/7 HTS kit.

KIT CONTENT

- 1) 1 vial Caspase 3/7 Reagent. (z-DEVD)₂ Rhodamine 110
- 2) 1 Bottle of Cell Lysis Buffer.

Ordering Information

Catalog #	Size	Price (US\$)
Apo200-2	100 Tests	275
Apo200-3	500 Tests	775
Apo200-4	1000 Tests	1395

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