



Apo 3/7 HTS™

High Throughput Screen for Caspase 3/7

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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 C α , actin and a 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.

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.

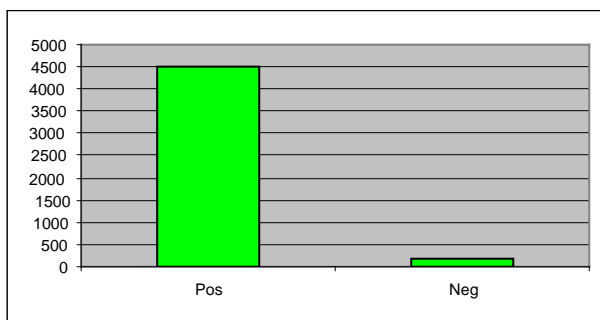


Figure 1. U937 cells were stimulated with 1 μ M staurosporine for 5 hours. After which caspase 3/7 activity was analyzed using the Apo 3/7 HTS kit.

Section A: Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. Practice safe laboratory procedures by wearing protective clothing and eye ware.
3. **Not** for Flow Cytometer or fluorescent imaging applications.

Section B: Storage and Shelf Life

Quenched (z-DEVD)₂ R110 Dye Reagent:

1. Store the lyophilized reagent at -20°C . Store with desiccant pouch.
2. Store the reconstituted reagent at -20°C .
3. Make the 2X working solution immediately before use. The 2X solution maybe stored for 24 hours at 4°C prior to use.
4. Lysis Buffer: Store at 25°C .

Section C: 100 Tests: Kit Content

1. 1 vial Caspase 3/7 detection reagent with desiccant pouch: Part# 4005
2. 1 Bottle 1X Lysis Buffer: 22mL Part# 3005

500 Tests: Kit Content

1. 5 vial Caspase 3/7 detection reagent with desiccant pouch: Part# 4005
2. 1 Bottle 1X Lysis Buffer: 110mL Part# 3005

Section D: Materials Required But Not Supplied

1. Tissue culture media
2. Black 96 well or greater (clear bottom optional) tissue culture plates.
3. Fluorescence Plate Reader with 488 nm excitation source and capable of measuring 515-530 nm (E.g. FITC filter set) emission.
4. DTT Sigma Cat# D0632

Section E: Experimental Preparation and Setup

2X Working Dilution of caspase 3/7 detection reagent

1. Reconstitute the lyophilized caspase detection with 400 μL of DMSO. This will make a **50X** solution.
2. Warm Lysis Buffer until it reaches room temperature. 100 μL of Lysis buffer is required per 100 μL sample.
3. Make a 1Molar stock solution of DTT, aliquate and freeze.

For example: 10 samples x 100 μL per sample = 1000 μL of Lysis Buffer required.

3. Add DTT to your required volume of lysis buffer so you have a 10-20mM final concentration. Smaller volume samples can be used as long as a 1:1 ratio of sample to lysis buffer is maintained.
4. The next step is to make a 2X concentrate of the caspase detection reagent. This can be accomplished by diluting the **50X** caspase reagent 1:25 into your required volume of lysis buffer. For example 1000 μL of Lysis Buffer required/25 (dilution)= 40 μL of caspase reagent required. So to 960 μL of Lysis Buffer add 40 μL of caspase reagent = 1000 μL . This equals a 2X concentrate caspase reagent.
5. Add 100 μL of the 2X caspase 3/7 detection reagent to 100 μL of sample.

Section F: Protocol: 96 Well ELISA Plate

1. Add 100 μ L of cell samples per well and induce apoptosis according to your experimental protocol. Also include a negative control at this time point, e.g. vehicle treated cells. Volumes may be adjusted according to the number of wells per plate.

2. After appropriate time point, add 100 μ L of the 2X working stock of caspase 3/7 detection reagent (as diluted above). Incubate the samples according to your experimental protocol (e.g. 37⁰C at 10%CO₂) for an additional 30 – 60 minutes.

Note: individual investigators should titrate out reagent to optimize staining concentration and length of incubation.

3. Measure fluorescence utilizing a plate reader with 488nm excitation and emission at 515-530nm.

Technical note#1

The assay can be scaled to meet a variety of sample volumes by maintaining a 1:1 ratio between the sample volume and dilution/lysis buffer which has the caspase 3/7 detection reagent add to it (Section E step 2 above).

Publications Cited in Manual

References

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