

## THE APO-ONE™ HOMOGENEOUS CASPASE-3/7 ASSAY: A SIMPLIFIED “SOLUTION” FOR APOPTOSIS DETECTION

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*Here we describe some of the many applications possible using the highly sensitive and robust Apo-ONE™ Homogeneous Caspase-3/7 Assay (Cat.# G7790, G7791). The engineered flexibility of the assay allows for broad implementation and miniaturization—from the life science researcher’s bench to high-throughput robotic platforms.*

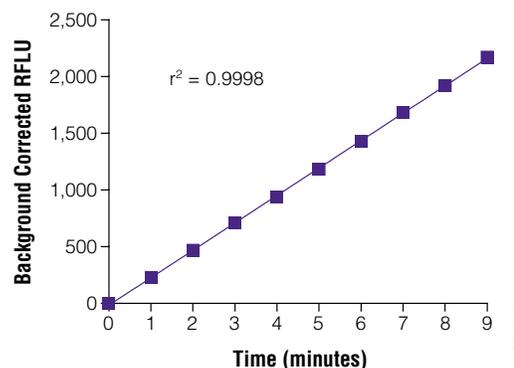
### Introduction

In 1972 Kerr *et al.* (1) coined the term apoptosis to describe an active cell death program in normal hepatocytes. Scientists studying apoptosis continue to work on achieving balance between improvements in throughput and sensitivity for detecting apoptosis, and the relative amount of information gained in deciphering apoptotic events. The characteristic morphological observations of cellular condensation, membrane blebbing and chromatin condensation (2) remain the gold standard but are often laborious to determine and poorly quantitative. Various immunohistochemical and DNA end-labeling analyses are definitive, but they require a certain degree of specialized proficiency and ancillary microscopic equipment.

The discovery of the cysteine aspartyl protease family of enzymes (caspases), which actively mediate apoptotic events, provided a major technological leap for apoptosis detection and quantitation. Standard caspase enzymatic cleavage assays that use fluorescently labeled synthetic tetrapeptide substrates can now be easily and routinely implemented as early indicators of apoptotic processes in high-throughput formats.

### Easier preparation and greater sensitivity

Promega has simplified and improved the traditional caspase assay by eliminating sample preparation steps and improving sensitivity. The Apo-ONE™ Homogeneous Caspase-3/7 Assay<sup>(a)</sup> uses a single-step reagent addition of a bi-functional lysis/activity buffer that contains an improved caspase substrate. The buffer allows rapid and efficient lysis while supporting optimal caspase-3/7 activity (Figure 1). The Z-DEVD-R110 substrate offers considerable advantage over Ac-DEVD-AMC or derivative fluors in terms of relative fluorescence (Figure 2). The signal-to-noise ratios with this system represent a 40% improvement over other systems.



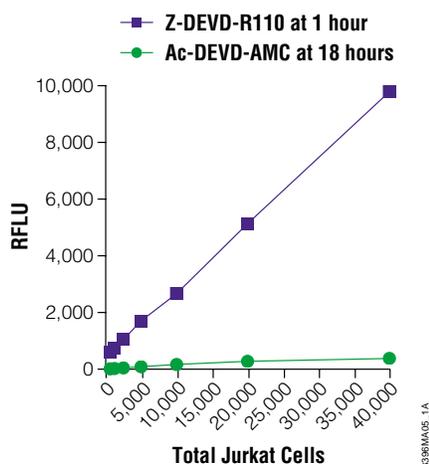
**Figure 1. Rapid and efficient cell lysis and optimal caspase activity are obtained with this assay.** Cellular lysis is almost instantaneous upon the addition of the Homogeneous Caspase-3/7 Reagent after gentle shaking. Fluorescent product generated by caspase-3/7 cleavage of the Z-DEVD-R110 substrate accumulates in a linear manner as a function of time.

### Ideal for high-throughput screening

Because apoptosis is often a consequence of toxicity, the development of novel therapeutic agents requires that researchers identify apoptotic induction early in drug development. Furthermore, in the search for effective cancer therapies, compounds that specifically induce apoptosis are potentially useful pharmaceuticals. Whether screening a biased chemical library for active lead compounds, culling potentially toxic substances, or defining limitations of gene delivery systems, rapid and sensitive detection of apoptosis is imperative. The Apo-ONE™ Homogeneous Caspase-3/7 Assay provides a one-step method that lends itself to automated platforms and high-throughput screening. This assay is easily conducted by adding candidate chemical entities to primary, suspension, or adherent cell cultures or purified enzyme preparations. After incubation with the candidate compound, the Apo-ONE™ Homogeneous Caspase-3/7 Assay Reagent is added and incubated, and caspase activity determined.

### Secondary screening of induction agents

Drug development protocols must determine the optimal apoptotic induction or therapeutic range for chemical or biological agents for a given model system. As in apoptotic identification above, this screening is necessary to define whether lead compounds meet certain established criteria for induction or inactivation of caspases. Researchers can use this information to determine if a compound merits further development or derivitization. Figure 3 illustrates data to determine the effective dosage (ED<sub>50</sub>) for the protein kinase C (PKC) inhibitory compound,



**Figure 2. Apoptosis induction in Jurkat cells via Fas receptor engagement over a 5-hour period.** Cells were serially diluted into a 96 well plate. Homogeneous Caspase-3/7 Reagent containing either Z-DEVD-R110 or Ac-DEVD-AMC was added to the cells and incubated at ambient temperature. Z-DEVD-R110 data were collected on a fluorometer 1 hour post-addition, whereas Ac-DEVD-AMC data were collected at 18 hours post-addition.

staurosporine. We serially diluted staurosporine and added it to cultured HeLa cells in a 96 well plate. After a 5-hour induction, the Apo-ONE™ Homogeneous Caspase-3/7 Assay Reagent was added to each well and incubated. The liberated fluor was detected using a plate-reading fluorometer.

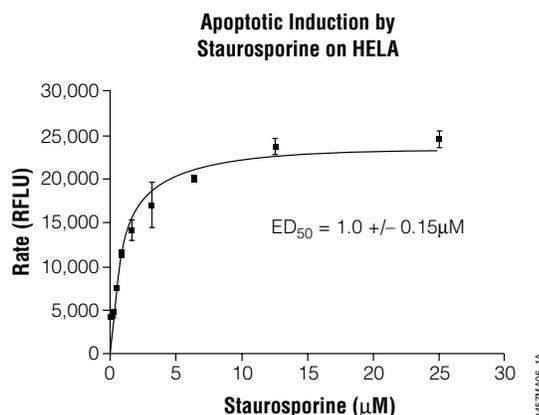
Inhibition constants ( $K_i$ ) are also easily obtained using this assay. In our model, we added serial dilutions of Ac-DEVD-CHO to a purified active caspase-3 preparation and allowed the inhibition to reach equilibrium over a 1-hour time period. We then added the Homogeneous Caspase-3/7 Reagent and determined the residual caspase activity present in each dilution of inhibitor (Figure 4).

### Summary

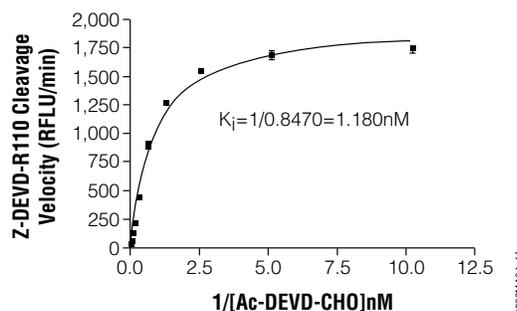
Promega has developed a simplified assay system for rapid, sensitive and efficient detection of caspase-3/7 activity in primary, adherent and suspension cell culture as well as in purified caspase solutions. The one-step assay reagent allows for immediate lysis of sample cells while supporting optimal caspase activity. The assay can be easily scaled and configured to meet the miniaturization needs of high-throughput screening or life science research applications, whether cell-based or with purified enzyme, while retaining exquisite sensitivity for caspase activity.

### References

1. Kerr, J.F. *et al.* (1972) *Br. J. Cancer* **26**, 239–257.
2. Strasser, A. *et al.* (2000) *Archives Biochem.* **69**, 217–236.



**Figure 3. Apoptosis ED<sub>50</sub> determination of staurosporine on HeLa cells.** Serial dilutions of staurosporine were made in culture media and added to monolayers of HeLa cells. After 5 hours of incubation, the Homogeneous Caspase-3/7 Reagent was added and incubated for an additional 2 hours prior to analysis on a fluorometer. The graph was generated and nonlinear regression performed using Prism® software ( $r^2=0.92$ ).



**Figure 4. Inhibition constant ( $K_i$ ) determination for Ac-DEVD-CHO against purified caspase-3.** Serial dilutions of the inhibitor Ac-DEVD-CHO were added to a 96 well plate. Purified caspase-3 was added to the plate and the reactions incubated for 1 hour at ambient temperature. We added the Homogeneous Caspase-3/7 Reagent to the plate and made kinetic measurements of residual caspase-3 activity. The graph was generated and nonlinear regression performed using Prism® software ( $r^2=0.9882$ ).

### Ordering Information

Product	Size	Cat.#
Apo-ONE™ Homogeneous Caspase™-3/7 Assay <sup>(a)</sup>	10ml	G7790
	100ml	G7791
Caspase Inhibitor Ac-DEVD-CHO	100μl	G5961

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