

## Apo-ONE™ Homogeneous Caspase-3/7 Assay: Robust, High-Throughput Apoptosis Detection

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### Abstract

*The biological importance of the apoptotic process has gained increased attention in recent years (1), ranging from general to specific analysis and encompassing fields of research as diverse as developmental biology and toxicology. However, limitations in a number of experimental methods for apoptosis detection have led to a compromise between apoptotic assay information content, complexity, throughput and rate of technical success. The advent of caspase-based proteolysis assays has opened the door to rapid and definitive confirmation of apoptotic events. Here we describe a simple, sensitive and flexible assay that is as robust and informative when automated with state-of-the-art robotics as it is when performed manually at the benchtop.*

**The Apo-ONE™ Homogeneous Caspase-3/7 Assay represents a fast and sensitive alternative to other more laborious and technically difficult methods of detecting apoptosis.**

### Introduction

Homeostasis in multicellular organisms is maintained to a large extent by a careful and coordinated deletion of cells no longer necessary for overall survival. This elimination, known as apoptosis, can be mediated by cell receptor signal transduction events based on antagonistic or agonistic ligand receptor interactions or by molecules affecting functions within the mitochondria (2). The resulting sequential, energy-dependent, enzymatic cascade involving proteolytic enzymes called caspases leads to destruction of integral intracellular DNA repair elements, structural polypeptides and signaling kinases. The primary proteolytic executioners in this process, caspases-3 and -7, are useful for the timely and reliable detection of apoptotic events. The Apo-ONE™ Homogeneous Caspase-3/7 Assay<sup>(a)</sup> provides a fast and sensitive alternative to other more laborious and technically difficult methods of detecting apoptosis.

### Apoptotic Applications Abound

The convenient and flexible format of the Apo-ONE™ Homogeneous Caspase-3/7 Assay allows for a multitude of experimental applications to investigate apoptosis. Recently, much effort has been dedicated to mechanistic dissection of system-specific apoptosis by use of targeted inhibitors or initiators to understand the processes relating to homeostasis and dysregulation. Identification of key control-point molecules and modulation of their activity is central to therapeutic interventions in

oncogenesis or neurodegenerative disorders. In addition to specific apoptotic applications, other more global attributes or causal relationships of treatments or compounds can also be quickly defined with high sensitivity in toxicological applications.

### Built-In Flexibility

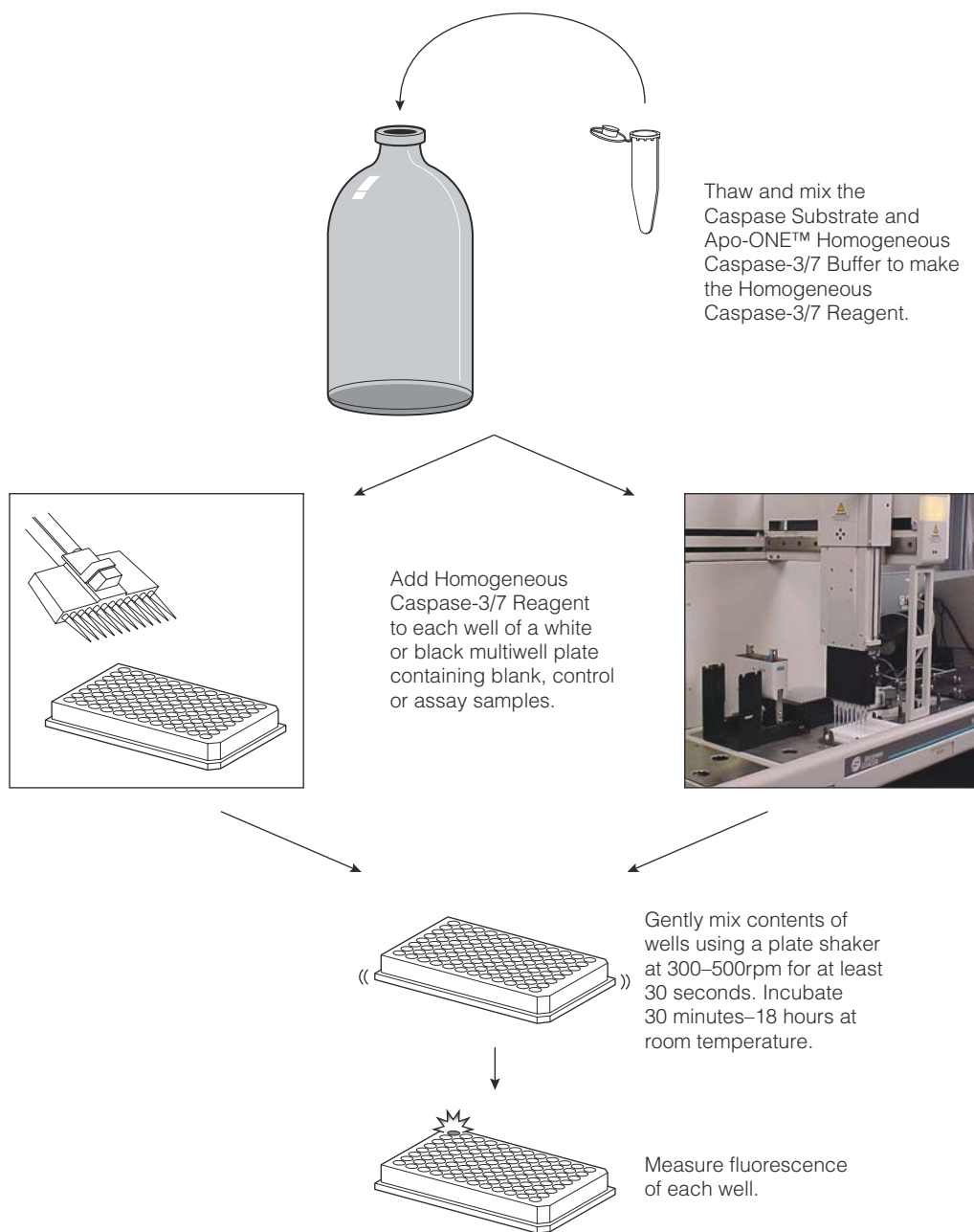
The Apo-ONE™ Homogeneous Caspase-3/7 Assay is designed to be simple and user-friendly, independent of the size of a given study. Whether preparing enough reagent for the analysis of a few samples or for screening a million-compound library, preparation of the working solution is simple. After the kit is removed from frozen storage and components thawed to room temperature, the Z-DEVD-Rhodamine 110 substrate is added to the homogeneous lysis/activity buffer at a ratio of 1:100 to create the Homogeneous Caspase-3/7 Reagent. The volume of reagent prepared is based upon the cell culture well volume and the total number of plates.

### Add, Mix and Measure

The Apo-ONE™ Homogeneous Caspase-3/7 Assay can be performed in two principle formats with varying scale. The reagent can be delivered either manually or by a robotic liquid-handler, as shown in Figure 1. Central to this protocol is a brief mixing step, which ensures sample/reagent homogeneity while facilitating cellular lysis/permeabilization and substrate access to the liberated caspases. After an incubation period that allows caspase-3 and -7 cleavage of the substrate and release of the fluorescent rhodamine 110 product, the plate(s) can be read in standard fluorometric fashion using conventional readers equipped with a 485±20nm excitation source and 530±25nm emission collector. Figure 2 shows the results of Apo-ONE™ Assays performed manually (Panel A) and with a Biomek® 2000 workstation (Panel B). Fluorescence values for Figure 2 are indicated in relative fluorescence units (RFU).

### Expanding the Assay's Information Content

Because of the unique spectral properties of the Apo-ONE™ Homogeneous Caspase-3/7 Assay's reporting fluor molecule (rhodamine 110; reference 3), preliminary studies indicate that multiplexing with other noninterfering fluorescent biological indicators is possible. It may be feasible, therefore, to include a fluorescent oxidation-reduction indicator (4) directly into treated cell populations to assess cell "health" prior to analysis with the Apo-ONE™ Assay. Figure 3 demonstrates the use of a fluorescent oxidation-reduction indicator dye with the Apo-ONE™ Assay to confirm decreased cell viability and



**Figure 1. Manual and robotic handling of reagents with the Apo-ONE™ Homogeneous Caspase-3/7 Assay.**

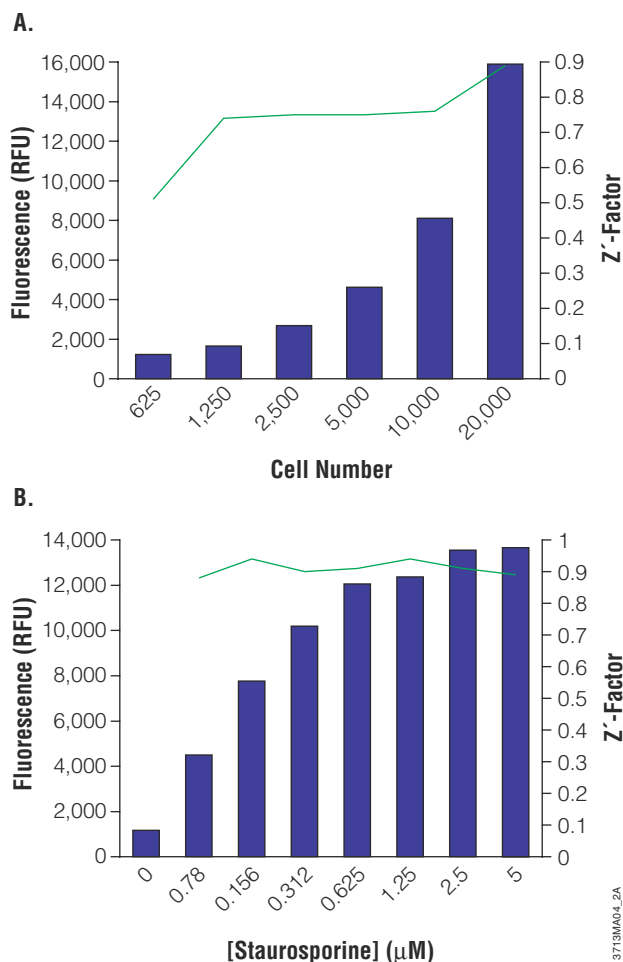
caspase induction. These output measures are likely to be inversely complementary in cell death processes mediated by apoptotic events.

## Conclusions

The Apo-ONE™ Homogeneous Caspase-3/7 Assay is a sensitive and rapid method for detecting caspase activity in cell culture or purified enzyme systems. Its inherent simplicity, flexibility and robustness allow for a myriad of apoptosis array applications both at the benchtop and for automated high-throughput analysis.

## References

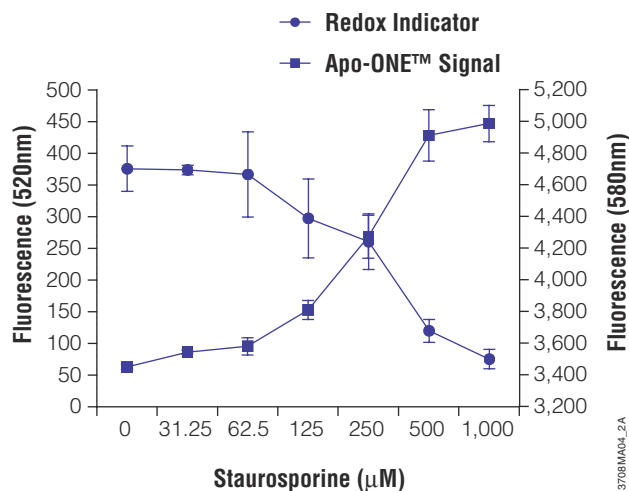
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3. Liu, J. *et al.* (1999) *Bioorg. Med. Chem. Lett.* **9**, 3231–3236.
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**Figure 2. The Apo-ONE™ Homogeneous Caspase-3/7 Assay Reagent has been qualified at Promega for manual and high-throughput applications in 96- and 384-well formats by an automated reagent delivery module (5).** The one-step Apo-ONE™ Reagent is reliably delivered in this manner, not hampered by viscosity or foaming. Z'-factor values (6) are a dimensionless statistical measure of signal strength and standard deviation. The Z'-factor values shown on these graphs indicate high quality and good reproducibility of data. In Panels A and B the bars show fluorescence data (left y axis) while the lines represent Z'-factor values (right y axis). **Panel A:** Two-fold serial dilutions of Jurkat cells were induced in a 96-well plate with 500nM staurosporine for 5 hours at 37°C with 5% CO<sub>2</sub>. The Apo-ONE™ Reagent was added with a multichannel pipette and incubated for 3 hours prior to measurement. **Panel B:** In a separate study, Jurkat cells were added to a 96-well plate at a concentration of 20,000 cells/well in 50μl volumes, then induced to undergo apoptosis with varying concentrations of staurosporine. After a 5-hour incubation, Apo-ONE™ Reagent was added using the Biomek® 2000 (Beckman Instruments; see Figure 1). The resulting fluorescence was measured after 3 hours.

## Protocol

- ◆ Apo-ONE™ Homogeneous Caspase-3/7 Assay Technical Bulletin #TB295, Promega Corporation.  
[www.promega.com/tbs/tb295/tb295.html](http://www.promega.com/tbs/tb295/tb295.html)



**Figure 3. Multiplexed, fluorescent assay yields inverse measures of cell viability and caspase activation.** Jurkat cells were added to an opaque 96-well plate at a concentration of 10,000 cells/well in 50μl volumes. Serial dilutions of staurosporine (50μl) were added to the cells, followed immediately by addition of a commercially available fluorescent redox indicator (10μl). Samples were mixed by orbital shaking and incubated for 4 hours at 37°C, 5% CO<sub>2</sub>. The plate was read at 530/580nm, then Apo-ONE™ Reagent was added, followed by a 1-hour incubation and a second measurement. Although Apo-ONE™ caspase-mediated fluorescence is significantly quenched (50–70%) by the presence of the redox indicator dye, sufficient remaining signal allows for multiplexing to confirm both reduced cell viability and the induction of caspases in cell culture. The redox indicator fluorescence is on the left y axis, the Apo-ONE™ fluorescence on the right y axis.

## Ordering Information

Product	Size	Cat.#
Apo-ONE™ Homogeneous Caspase-3/7 Assay <sup>(a)</sup>	1ml	G7792
	10ml	G7790
	100ml	G7791
Apo-ONE™ Homogeneous Caspase-3/7 Buffer	100ml	G7781

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