

Apo-ONE™ Homogeneous Caspase-3/7 Assay: A Rapid, Sensitive and Flexible Apoptosis Detection System

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The Apo-ONE™ Homogeneous Caspase-3/7 Assay^(a) is a revolutionary improvement in apoptosis detection methodology. Promega's proprietary lysis/activity buffer, in conjunction with the (Z-DEVD)₂-Rhodamine 110 substrate, enables a simple "add-mix-read" format for the detection of caspase-3 and -7 in adherent, suspension, and primary culture cells, or in purified caspase preparations. The homogeneous format eliminates tedious washing, concentration, and multiple freeze-thaw steps required by conventional caspase detection assays, resulting in a dramatic reduction in sample preparation time. The rhodamine 110-based substrate allows for exquisite sensitivity previously unobtainable with conventional colorimetric or fluorometric assays. Furthermore, the assay is inherently flexible for use in a variety of volumes and formats from cuvettes to 384 well plates.

Advantages of the Apo-ONE™ Homogeneous Caspase-3/7 Assay

Convenient: The Homogeneous Caspase-3/7 Reagent is made simply by diluting the substrate 1:100 with the assay buffer.

Fast: Minimal time is required for sample preparation and for generation of statistically relevant data. One hour of incubation with the Homogeneous Caspase-3/7 Reagent and cultured cells is typically sufficient, although longer incubations are not detrimental and may benefit signal-to-noise ratios.

Flexible: Assay volume is flexible as long as the 1:1 ratio of reagent volume to sample volume is preserved. The assay can be performed in a cuvette, 96 or 384 well plate. Furthermore, data can be collected within one hour and for up to 18 hours after Reagent addition.

Sensitive: Detects significant caspase-3/7 activity in low numbers of total cells (cell numbers in the hundreds). Longer incubations can be used to obtain results in systems using low numbers of cells or in cells that express low levels of caspase-3/7.

Informative: Defines mechanistic toxicity. The assay can be used to conveniently assess apoptotic induction or inhibition after cell culture treatment.

Automatable: The assay's "add-mix-read" format can be easily integrated into automated platforms such as the Biomek® 2000 and Biomek® FX (Beckman).

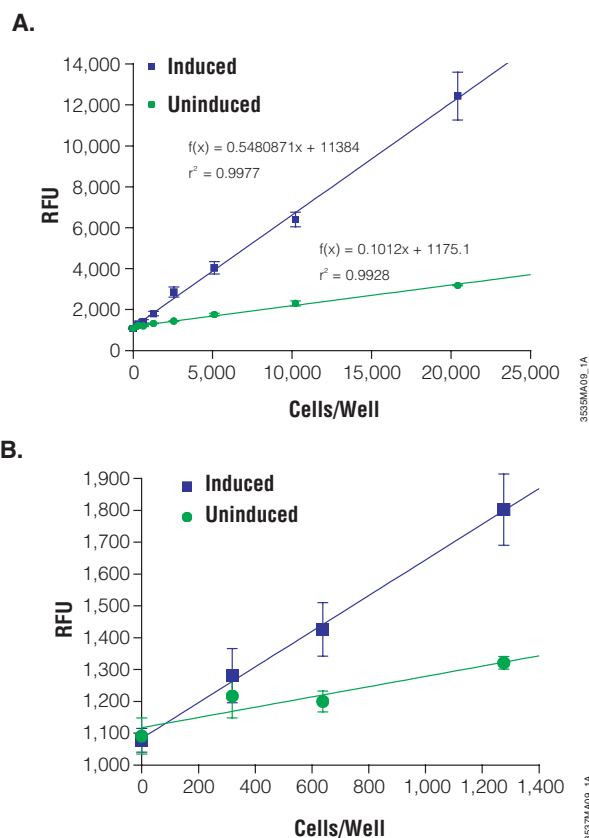


Figure 1. Sensitivity of the Apo-ONE™ Homogeneous Caspase-3/7 Assay in a neuroblastoma cell line. Panel A. SH-SY5Y cells were serially diluted in MEM Eagle's + F-12 medium containing 10% fetal bovine serum and transferred into a sterile Costar® 96 well plate. The cells were allowed to adhere overnight in an incubator at 37°C with 5% CO₂. Apoptosis was induced with 500nM staurosporine during a 6-hour incubation. Uninduced cells were treated with comparable levels of vehicle. After induction, active caspase-3/7 was assayed using the method provided in the *Apo-ONE™ Homogeneous Caspase-3/7 Assay Technical Bulletin*, #TB295. Fluorescence was measured with the Cytofluor® II, fluorescent plate reader. **Panel B.** Sensitivity for this particular model of apoptosis is at least 638 cells/well after one hour in Homogeneous Caspase-3/7 Reagent.

Ordering Information

Product	Size	Cat. #
Apo-ONE™ Homogeneous Caspase-3/7 Assay ^(a)	10ml	G7790
	100ml	G7791
Caspase Inhibitor Z-VAD-FMK	50µl	G7231
	125µl	G7232

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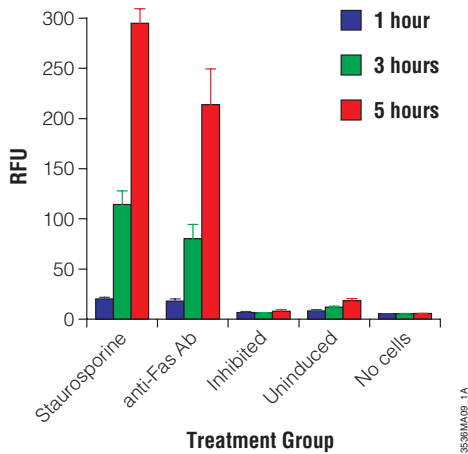


Figure 2. Increasing incubation time increases assay sensitivity. Jurkat cells were plated at a density of 5.0×10^3 cells/well of a 384 well plate. Induced cells were treated with 312nM staurosporine or 200ng/ml of anti-Fas antibody. Control cells were treated with vehicle alone. For our inhibited samples, we incubated the cells with Z-VAD-FMK at a final concentration of 20 μ M for 30 minutes prior to treating the cells with 200ng/ml anti-Fas antibody. Apoptosis was induced during a 5-hour incubation at 37°C and 5% CO₂. The Homogeneous Caspase-3/7 Reagent was added in a 1:1 ratio of caspase reagent:cultured cells in medium. Fluorescence was measured at various time intervals on the Fluoroskan plate reader. By increasing incubation time, we are able to detect caspase-3/7 activity with few cells or with cells that express low levels of caspase-3/7.

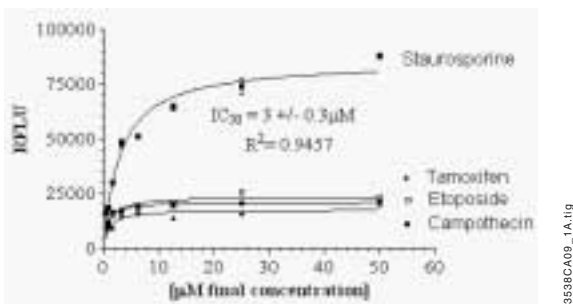


Figure 3. Dose response curves are easily obtained after cell treatment. Primary rat E18 striatal neural cultures were prepared and cultured as described by Learish *et al.* (1). Cells were seeded at 25,000 cells/well in Microtiter® plates and allowed to attach. Serial dilutions of staurosporine, tamoxifen, etoposide, and camptothecin were added to the culture and were incubated for a period of 4 hours. Homogeneous Caspase-3/7 Reagent was added, and the plates were incubated at room temperature, and read after an additional 3 hours of incubation. IC₅₀ data were plotted using conventional software (Prism®). (IC₅₀, induction concentration).

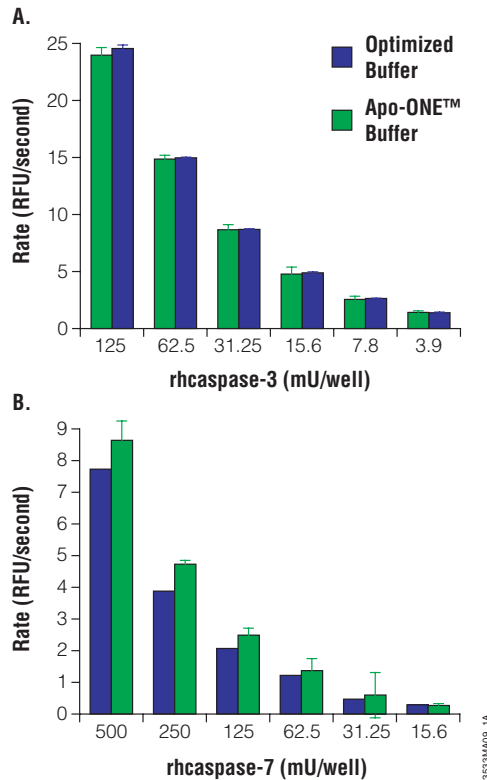


Figure 4. Apo-ONE™ Assay Buffer supports maximal caspase-3 and -7 activities. Panel A. Active recombinant human caspase-3 was two-fold serially diluted in either Homogeneous Caspase-3/7 Assay Buffer or the optimized buffer described in reference 2. An additional equal volume of respective buffers containing the caspase substrate (Z-DEVD)₂-R110 was added to each well, mixed, and kinetic measurements were made over 10 minutes on a fluorometer. Panel B. Active recombinant human caspase-7 was two-fold serially diluted in either Homogeneous Caspase-3/7 Assay Buffer or the optimized buffer described in reference 3. An equal volume of respective buffers containing the caspase substrate (Z-DEVD)₂-R110 was added to each well, mixed, and kinetic measurements were made over 10 minutes on a fluorometer.

References

- Learish, R.L., Bruss, M.D., and Haak-Frendscho, M. (2000) *Dev. Brain Res.* **122**, 97–109.
- Nicholson, D.W. *et al.* (1995) *Nature* **376**, 37–43.
- Garcia-Calvo, M. (1999) *Cell Death Differ.* **6**, 362–9.

Protocol

Apo-ONE™ Homogeneous Caspase-3/7 Assay Technical Bulletin #TB295

www.promega.com/tbs/tb295/tb295.html