Measuring Fluorescence Using the Apo-ONE® Homogeneous Caspase-3/7 Assay with the GloMax® Discover System

Promega Corporation

Materials Required

- Apo-ONE® Homogeneous Caspase-3/7 Assay (Cat.# G7790, G7791 and G7792)
- GloMax® Discover System (Cat.# GM3000)
- 96-well white or black plate suitable for cell culture

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin* #TB295 is available at:
www.promega.com/protocols/

Scientists studying apoptosis continue to work on achieving balance between improvements in throughput, sensitivity for detecting apoptosis and the relative amount of information gained in deciphering apoptotic events. 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. Promega has further simplified and improved the traditional caspase assay by eliminating sample preparation steps and improving sensitivity. The Apo-ONE® Homogeneous Caspase-3/7 Assay used in combination with the GloMax® Discover System provides a convenient, rapid and sensitive procedure for measuring cell apoptosis through fluorescence quantitation of Caspase-3/7 enzyme activity.

The Apo-ONE® Homogeneous Caspase-3/7 Buffer rapidly and efficiently lyses/permeabilizes cultured mammalian cells and supports optimal caspase-3/7 enzymatic activity. The caspase-3/7 substrate rhodamine 110, bis-(N-CBZL-aspartyl-L-glutamyl-L valyl-L-aspartic acid amide; Z-DEVD-R110), exists as a profluorescent substrate prior to the assay. To perform the Apo-ONE® Homogeneous Caspase-3/7 Assay, the Buffer and Substrate are mixed and added to the sample. Upon sequential cleavage and removal of the DEVD peptides by caspase-3/7 activity and excitation at ~499nm, the rhodamine 110 leaving group becomes intensely fluorescent with an emission maximum of 521nm.

The Apo-ONE® Homogeneous Caspase-3/7 Assay is made easy on the GloMax® Discover System. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows the user to easily measure various sample signal intensities on the same plate. Using the GloMax® Discover System, data are collected using a standard 96- or 384-well plate, and fluorescent output is correlated to caspase-3/7 concentration (Figure 1). This Application Note describes the protocol for measuring fluorescence using the GloMax® Discover System with the Apo-ONE® Homogeneous Caspase-3/7 Assay.

Detection of Caspase-3/7 Activity in Cell Culture (96-well, 200µl final reaction volume)

For detailed instructions and assay notes, see the *Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin* #TB295. The following procedure can be used for caspase-3 and -7 detection in cultured cells in a 96-well plate format but can easily be adapted for measuring caspase activity in purified caspase preparations as well as for 384-well format.

1. Thaw the 100X Substrate and Buffer to room temperature. Mix by inversion or vortexing. Dilute the Substrate 1:100 with the Buffer to obtain the desired volume of Apo-ONE® Caspase-3/7 Reagent.
2. Add 100µl of Apo-ONE® Caspase-3/7 Reagent to each well of a white or black 96-well plate containing 100µl of blank, control or cells in culture. Do not mix by manual pipetting.
3. Gently mix contents of wells using a plate shaker at 300–500rpm from 30 seconds up to read time. Incubate at room temperature for 30 minutes to 18 hours depending upon expected level of apoptosis (and thus caspase-3/7 activity) in the cells analyzed. The optimal incubation period should be determined empirically.
4. Measure the fluorescence of each well on the GloMax® Discover System (475_{Ex}/500–550_{Em}) within 18 hours.

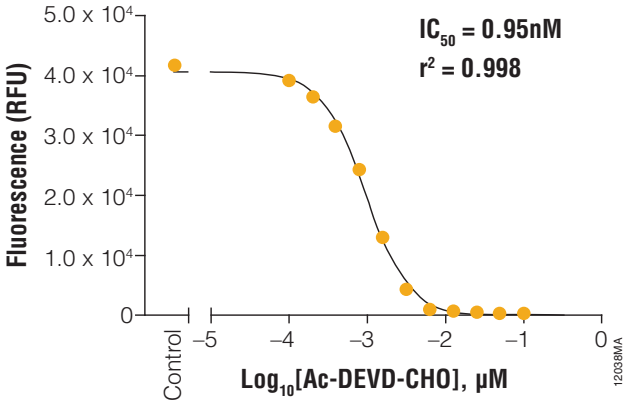

Figure 1. IC_{50} determination for Ac-DEVD-CHO. Serial dilutions of the Caspase Inhibitor Ac-DEVD-CHO (Cat.# G5961) were mixed with caspase-3 at 2U/ml (Enzo, Cat.# BML-SE169) and allowed to reach equilibrium in a final volume of 100µl by mixing on a plate shaker for 30 minutes. Apo-ONE® Homogenous Caspase-3/7 Reagent was added to the system, and activity rates were measured by reading fluorescence on the GloMax® Discover System (475_{Ex}/500–550_{Em}). To generate this plot, we performed nonlinear regression analysis on the data using GraphPad Prism® Software. Data points represent the average and standard deviation from triplicate samples.

GloMax® Discover System

The GloMax® Discover System was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System also provides flexible use of filters for fluorescence intensity, filtered luminescence, BRET, FRET, and UV-visible absorbance measurements for adaptation into a wide variety of laboratory applications. The instrument is operated by an integrated tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network. Thus together with the Apo-ONE® Homogenous Caspase 3/7 Assay, the GloMax® Discover offers a complete and easy to use solution to measure caspase activity.

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