Using the RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay on the GloMax® Discover System

Promega Corporation

Materials Required:

- RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay (Cat.# JA1011)
- GloMax® Discover System (Cat.# GM3000)
- Tissue culture incubator
- Cells and cell growth medium
- Recombinant human TRAIL (rhTRAIL)
- White, TC-treated 96-well assay place (Corning #3917 or 3903)

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

Protocols: RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay Technical Manual #TM507 and the GloMax® Discover System Technical Manual #TM397 available at: **www.promega.com/protocols/**

Distinct forms of programmed cell death (PCD) are important for maintaining homeostasis in multicellular organisms. For instance, PCD terminates or helps remove cells compromised by viral infections or toxins, as well as those harboring unrepairable DNA damage. The apoptotic process is particularly important for PCD and disruption has been associated with several disease states such as cancer, autoimmunity and neurodegenerative disorders. Therefore, modulation of the apoptotic process by therapeutic intervention offers hope for new and efficacious treatments.

Although the apoptotic process can be studied in vitro by several different techniques, most methods are either labor intensive or suffer from the unpredictability of the differential kinetics of induction. The RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay measures the exposure of phosphatidylserine (PS) on the outer leaflet of cell membranes during the apoptotic process. The assay is non-lytic and the simple "add-and-read" method allows multiple readings from a single assay well. In addition, the assay contains a Necrosis Detection Reagent which provides a real-time measure of cells that have progressed to secondary necrosis. The assay readout can be measured on the GloMax® Discover System, which provides extended dynamic range and superior sensitivity. This application note describes a protocol to measure apoptosis in real-time using the RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay kit and the GloMax® Discover System.

RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay

1. Plate 50µl of cells (U937 cells in this example) in growth medium (RPMI-1640 + 10% FBS) into each well of a white, TC-treated 96-well plate (10,000 cells/well). Include a set of control wells with no cells present (growth medium only) to determine background luminescence and background fluorescence.
2. Perform a 10-point 3-fold serial dilution of rhTRAIL (an extrinsic apoptosis inducer) in growth medium at 4x the desired final concentration. Be sure to include a set of control wells with no rhTRAIL present (untreated controls). Add 50µl of the 10-point 3-fold serial dilution of rhTRAIL (and no compound control) to the appropriate replicate wells in the 96-well assay plate.
3. Add 100µl of 2x concentrated RealTime-Glo™ Annexin V Apoptosis and Necrosis Detection Reagent in growth medium to each well.
4. Incubate cells in the covered 96-well assay plate at 37°C/5% CO_2 in a humidified cell culture incubator.
5. Measure luminescence and fluorescence (Ex 475, Em 500-550) on the GloMax® Discover instrument using the RealTime-Glo™ Annexin V Apoptosis Assay pre-programmed protocol at 0, 3.5, 6.5, and 24 hours.

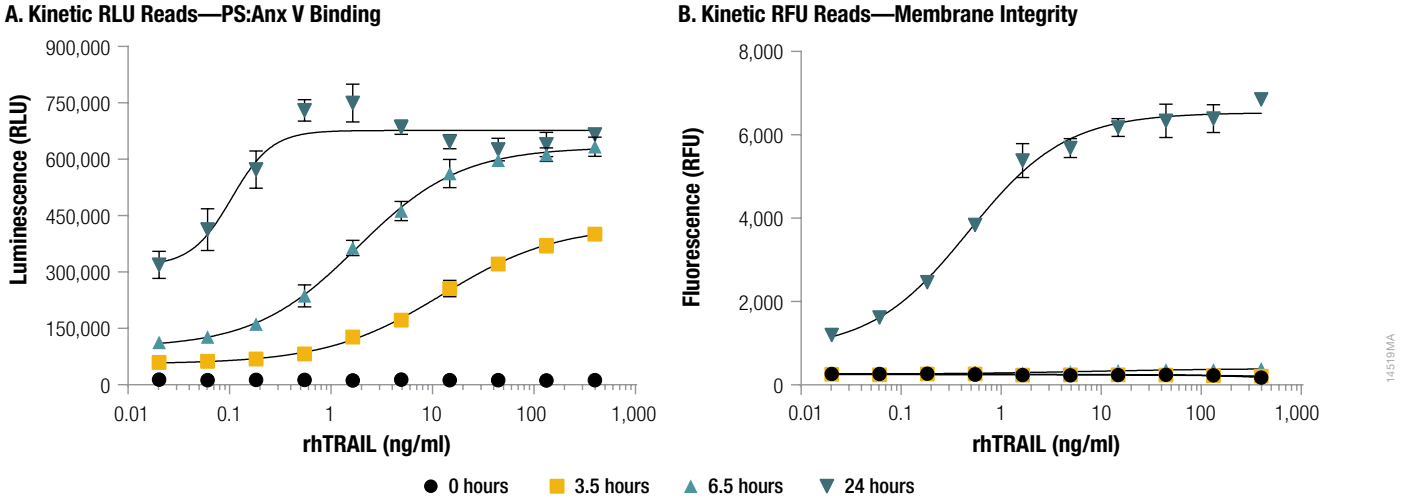

Figure 1. Annexin V binding to phosphatidylserine (PS:Anx) and loss of membrane integrity in real-time. U937 cells were incubated at 37°C/5% CO_2 in the presence of serial dilutions of rhTRAIL and the RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay Reagents. Background subtracted luminesecence, RLU (Annexin V binding to phosphatidylserine, panel A) and background subtracted fluorescence, RFU (membrane integrity, panel B) were measured at 0, 3.5, 6.5, and 24 hours on the GloMax® Discover instrument. The time-dependent increase in luminescence (due to Annexin V binding to phosphatidylserine, panel A) that occurs prior to the time-dependent increase in fluorescence (due to loss of membrane integrity, panel B) reflects apoptosis followed by secondary necrosis.

Conclusion

Detecting the real-time kinetics of apoptosis and secondary necrosis in vitro using a plate reader has been made possible by combining the simple homogeneous RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay kit and the GloMax® Discover System. The luminescent and fluorescent readouts can be measured repeatedly from the same samples over a long-term period to record the kinetics of different cell death processes. The GloMax® pre-programmed instrument protocol, instrument sensitivity and dynamic range performance provide a robust system for measuring programmed cell death in real time.

