# Measuring Fluorescence Using the ApoLive-Glo™ Multiplex Assay with the GloMax® Discover System

*Promega Corporation*

**Materials Required**

- ApoLive-Glo™ Multiplex Assay (Cat.# G6410, G6411)
- GloMax® Discover System (Cat.# GM3000)
- white 96-well plates

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

**Protocols:** *GloMax® Discover System Technical Manual* #TM397 and *ApoLive-Glo™ Multiplex Assay Technical Manual* #TM325 are available at: **www.promega.com/protocols/**

The ApoLive-Glo™ Multiplex Assay combines two Promega assay chemistries to assess viability and caspase activation events within a single assay well. When used with the GloMax® Discover System the ApoLive-Glo™ Multiplex Assay provides a convenient, rapid and sensitive procedure, using both fluorescent and luminescent quantitation to determine specific cellular physiological conditions.

The ApoLive-Glo™ Multiplex Assay combines two assay chemistries to assess viability and caspase activation events within a single assay well. The first part of the assay measures the activity of a protease marker of cell viability. The live-cell protease activity is restricted to intact viable cells and is measured using a fluorogenic, cell-permeant peptide substrate (glycyl-phenylalanyl-amino fluorocoumarin; GF-AFC). The substrate enters intact cells, where it is cleaved by the live-cell protease activity to generate a fluorescent signal proportional to the number of living cells. The live-cell protease becomes inactive upon loss of cell membrane integrity and leakage into the surrounding culture medium. The second part of the assay uses the Caspase-Glo® Assay technology to detect caspase-3/7 activation, which is a key biomarker of apoptosis. The Caspase-Glo® 3/7 Assay provides a luminogenic caspase-3/7 substrate, which contains the tetrapeptide sequence DEVD, in a reagent optimized for caspase activity, luciferase activity and cell lysis. Adding the Caspase-Glo® 3/7 Reagent in an “add-mix-measure” format results in cell lysis, followed by caspase cleavage of the substrate and generation of a “glow-type” luminescent signal produced by luciferase. Luminescence is proportional to the amount of caspase activity present.

The ApoLive-Glo™ Multiplex 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 allow the user to easily measure various sample signal intensities on the same plate. Fluorescence and luminescence readings are collected using the GloMax® Discover System (Figure 1). This Application Note describes the protocol for measuring fluorescence using the GloMax® Discover System with the ApoLive-Glo™ Multiplex Assay.

## Protocol

For detailed instructions and assay notes, follow the procedure in the *ApoLive-Glo™ Multiplex Assay Technical Manual*, #TM325. The following procedure can be used for measuring cell viability and cytotoxicity in a 96-well plate format.

### Storage and Preparation of Reagents

1. Thaw the Assay Buffer and GF-AFC Substrate in a 37°C water bath. Thaw the Caspase-Glo® 3/7 Buffer and Caspase-Glo® 3/7 Substrate at room temperature.
2. Transfer 10µl of GF-AFC Substrate into 2ml of Assay Buffer. Mix the Assay Buffer containing substrates by vortexing the contents until thoroughly dissolved. This mixture will be referred to as the Viability Reagent.
3. Transfer the contents of one Caspase-Glo® 3/7 Buffer bottle into one amber bottle containing Caspase-Glo® 3/7 Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to form the Caspase-Glo® 3/7 Reagent.

### Sample Assay Protocol for 96-Well Plate Format

1. Set up 96-well assay plates containing cells in medium at the selected density (we recommend using <20,000 cells per well).
2. Add test compounds and vehicle controls to appropriate wells for a final volume of 100µl per well.
3. Culture cells for the desired test exposure period. **Note:** In vitro cytotoxicity is dependent upon compound dosage and cell exposure period. Inappropriate exposures may result in misleading compound profiles. Therefore, we recommend characterizing new compounds in multiple exposure periods (4, 12, 24 and 48 hours) to determine the mechanism of cell death.
4. Add 20µl of Viability Reagent to all wells and briefly mix by orbital shaking (300–500rpm for ~30 seconds).
5. Incubate for at least 30 minutes (and no longer than 3 hours) at 37°C.

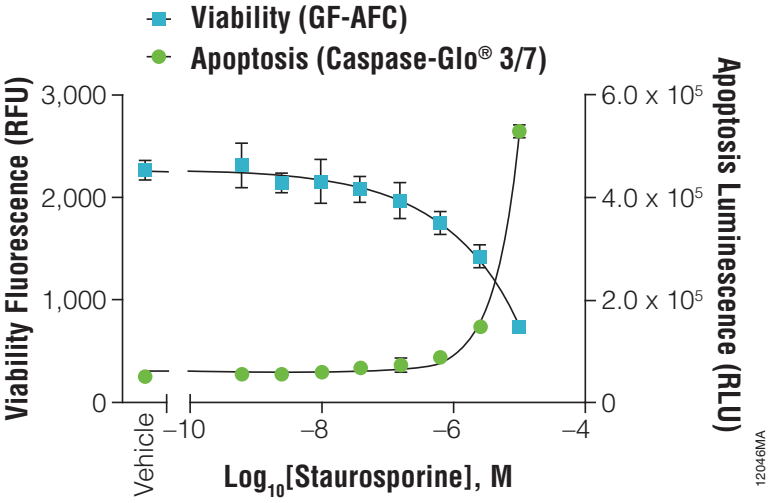


**Figure 1. Sample assay data from the ApoLive-Glo™ Multiplex Assay on the GloMax® Discover System.** The Viability Reagent was added to wells and viability measured after incubation for 40 minutes at 37°C. Caspase-Glo® 3/7 Reagent was added and luminescence measured after a 40-minute incubation at room temperature (10,000 HEK293 cells/well in a 96-well plate). Staurosporine treatment for 6 hours should result in a dose-dependent decrease in cell viability and an increase in caspase-3/7 activity, consistent with apoptosis.

6. Measure fluorescence on the GloMax® Discover System ($405nm_{Ex}$/$495\text{-}505nm_{Em}$).
7. Add 100µl of Caspase-Glo® 3/7 Reagent to all wells, and briefly mix by orbital shaking (300–500rpm for approximately 30 seconds).
8. Measure luminescence on the GloMax® Discover System.

### GloMax® Discover System

The GloMax® Discover System offers superior sensitivity, dynamic range and limited well-to-well cross talk. The instrument 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 to your local network.