

# Homogeneous Luminescent Caspase Assays to Detect Apoptosis & *In Vitro* Toxicity

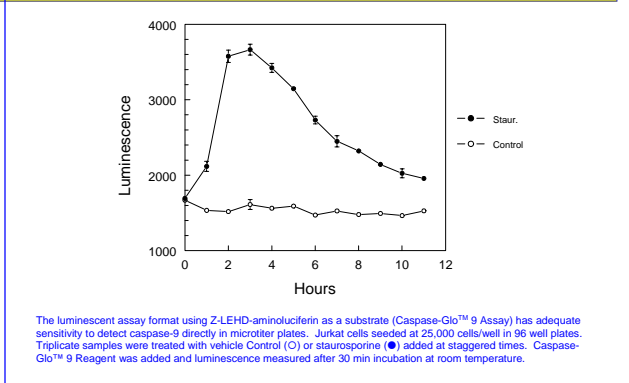
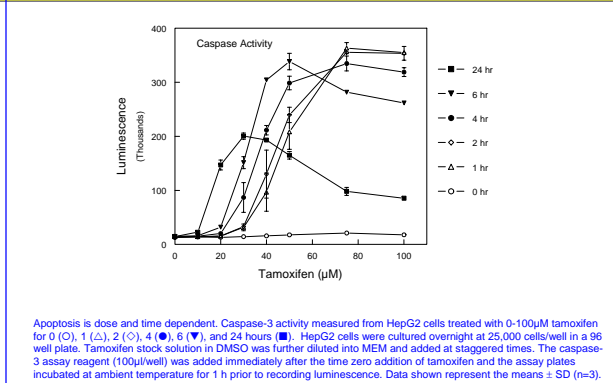
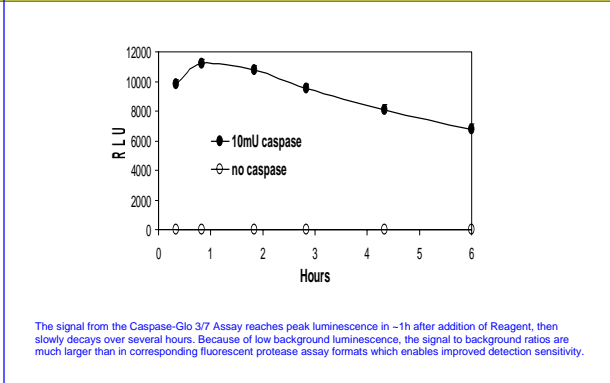
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Abstract	The Luminescent Signal Glows for Hours	Caspase-Glo 3/7 Time Course	Staurosporine Induction of Caspase-9 in Jurkat Cells
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**Abstract**

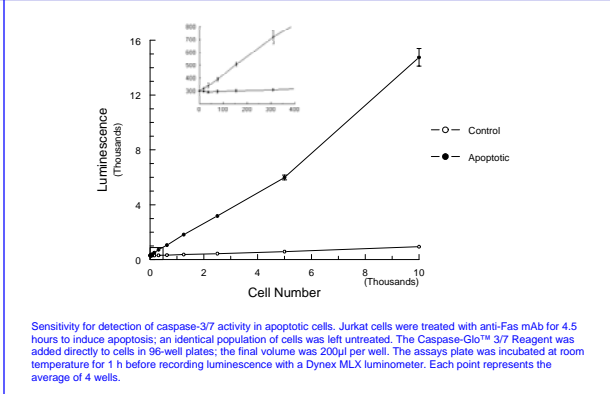
We have developed a homogeneous luminescent method to measure protease activity. Luminescent substrates have been developed for the measurement of caspase-3/7, -8, & -9 activities as *in vitro* markers of apoptosis as well as for screening for caspase inhibitors. The homogeneous format of the Caspase-Glo™ Assays was enabled by utilizing a mutant form of beetle luciferase that is stable in the conditions necessary to lyse cells and maintain caspase activity for hours. The Caspase-Glo™ Assay procedure is to add reagent directly to cells in multiwell plates, incubate for 30 min-1 h and record luminescence. There is a linear relationship between the luminescent signal and caspase activity. The luminescent format is 50-fold more sensitive than fluorescent assays for detection of recombinant caspase-3 and can detect as few as 20 apoptotic cells. The stability of the reagents and glow-type luminescent signal are compatible with automation for HTS. Multiplexing of luminescent and fluorescent caspase assays is possible.



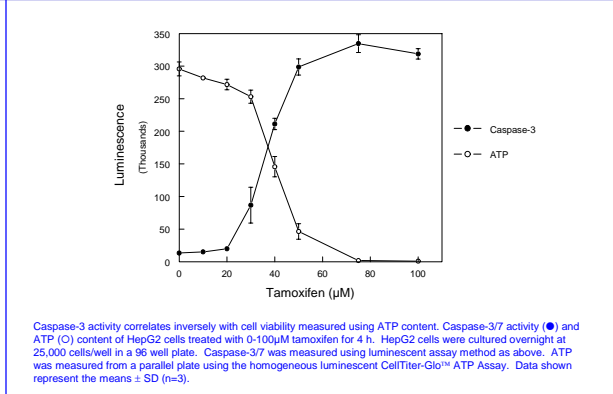
**Luminogenic Protease Assay Mechanism**

- Peptide-aminoluciferin conjugate is not a substrate for luciferase
- Caspase-3 cleaves peptide bond releasing aminoluciferin
- Aminoluciferin is now available as a substrate for luciferase
- Light is generated proportional to caspase activity

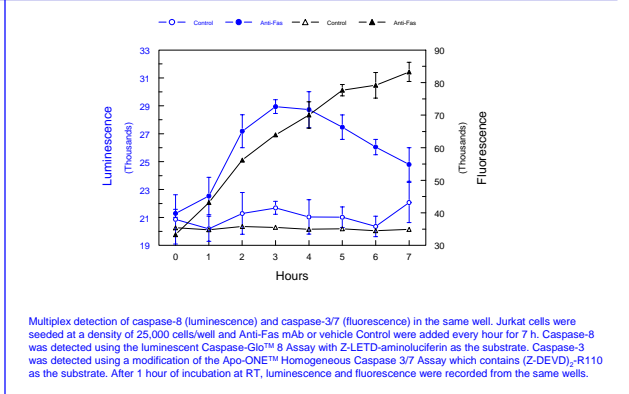
**Linearity & Sensitivity**



**Inverse Correlation of Caspase-3/7 and ATP Content**



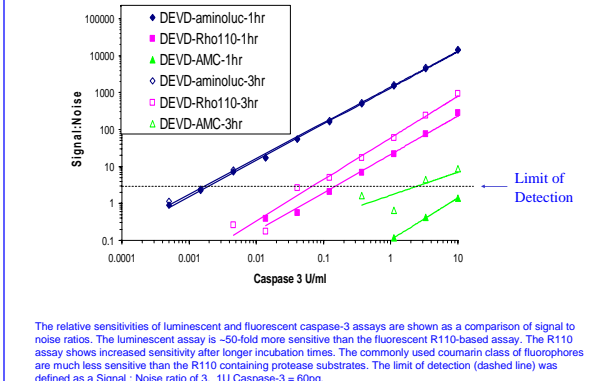
**Multiplexing Luminescent & Fluorescent Assays**



**Homogeneous Protocol to Measure Protease Activity**

- Prepare Reagent by adding Buffer to Lyophilized Substrate
- Equilibrate assay plate to 22°C
- Add Reagent directly to assay wells (1:1 vol)
- Incubate 30 min - 1 hour
- Record Luminescence

**Comparison of Luminescent & Fluorescent Sensitivity**



**Luminescence Shows Less Interference than Fluorescence**

**Summary of LOPAC Screens for Caspase-3 Inhibitors**

Luminescent Assay	Fluorescent Assay
6 hits (≥2 S.D. below mean)	9 hits (≥2 S.D. below mean)
1 of these was also a hit in fluorescent assay	1 of these was also a hit in luminescent assay
Remaining 5 are false hits (luciferase inhibitors)	Remaining 8 are false hits (fluorescence interference)
5/640 false hit rate = 0.78%	8/640 false hit rate = 1.3%

The luminescent Caspase-Glo 3/7 Assay and the Fluorescent Apo-ONE Homogeneous Caspase-3/7 Assay were used to screen for caspase-3 inhibitors present in the Sigma Library of Pharmacologically Active Compounds (LOPAC). The results indicated there was a lower frequency of interference with the luciferase-based protease assay compared to the fluorescent assay format.

**Summary**

- Homogeneous luminescent assays for caspase-3/7, -8, and -9 have been developed using peptide-aminoluciferin substrates.
- The luminescent signal is directly proportional to caspase activity and the number of apoptotic cells.
- The luminescent assay format is ~50-fold more sensitive than fluorescent protease assay methods.
- The Caspase-Glo™ Assays can detect caspase activity in the number of apoptotic cells typically cultured in microtiter plates.
- There is less chemical interference with the luminescent assay format compared to the fluorescent assay.
- The glow signal provides convenience for robotic HTS applications.
- Caspase-3/7 activity correlates inversely with ATP content viability assays and can be used to gather information about the type of cell death.
- Multiplexing of luminescent and fluorescent protease assays in the same well is possible
- For additional technical information, see <http://www.promega.com>