# DETECTING CASPASE ACTIVITY IN STAUROSPORINE-TREATED HUMAN NEUROBLASTOMA CELLS USING FLUORESCENT AND LUMINESCENT METHODS

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We used the Apo-ONE® Homogeneous Caspase-3/7 Assay and the Caspase-Glo™ 3/7 Assay to detect caspase-3/7 activity in human neuroblastoma SH-SY5Y cells. Caspase-3/7 activity was measured at various time points after adding assay reagents to SH-SY5Y cells treated with staurosporine. Optimal sensitivity for detection of caspase-3/7 activity was reached at 18 hours with the Apo-ONE® Homogeneous Caspase-3/7 Assay and at 1 hour with the Caspase-Glo™ 3/7 Assay. Both assays proved to be sensitive methods for measuring caspase-3/7 activity in SH-SY5Y neuroblastoma cells.

## Introduction

The human SH-SY5Y cell line is a subclone of the neuroblastoma cell line, SK-N-SH (1,2). This cell line is useful as an experimental model of neuronal apoptosis (3,4), as well as in the study of differentiation and neuritogenesis (5). Staurosporine induces apoptosis and caspase activation through a mitochondrion-mediated pathway (6). During apoptosis, cytochrome c is released from the mitochondria and activates caspase-9, which in turn activates caspase-3 (6). Caspase-3 plays a key effector role in apoptosis by cleaving specific substrates important for downstream apoptosis signaling. We demonstrate quantitative detection of caspase-3/7 activation in the SH-SY5Y cell line using the Apo-ONE® Homogeneous Caspase-3/7 Assay and the Caspase-Glo™ 3/7 Assay.

**Both the Apo-ONE® Homogeneous Caspase-3/7 Assay and the Caspase-Glo™ 3/7 Assay provide easy, rapid and sensitive methods for measuring caspase-3/7 activity.**

## Assay Principles

The Apo-ONE® Homogeneous Caspase-3/7 Assay[a] uses a proprietary lysis/activity buffer combined with $(Z\text{-DEVD})_2$-Rhodamine 110 substrate (7). This profluorescent substrate is cleaved by active caspase-3/7, producing the detectable fluorescent rhodamine 110 leaving group. Fluorescence can be measured using a standard fluorescent plate reader and is proportional to caspase-3/7 activity.

The Caspase-Glo™ 3/7 Assay[a,b] uses a proluminescent caspase-3/7 Z-DEVD-aminoluciferin substrate and a stable luciferase in a proprietary buffer optimized for caspase-3/7 activity, luciferase activity and cell lysis (8). Following caspase-3/7 cleavage of the pro luminescent substrate, a "glow-type" luminescent signal is generated by luciferase and can subsequently be measured using a standard luminometer.

Both of these systems enable homogeneous, rapid, and sensitive measurement of caspase-3/7 activity using an "add-mix-read" format.

## Methods

SH-SY5Y cells were cultured in 45% DMEM/45% F12-K medium supplemented with 10% fetal bovine serum at 37°C in 5% $CO_2$. Cells were plated at $1 \times 10^4$ cells per well in white or black clear-bottom 96-well tissue culture plates and incubated overnight. Cells were then treated for 5 hours with 1µM staurosporine or DMSO vehicle in the presence or absence of 20µM Z-VAD-FMK caspase inhibitor (n = 8).

Following induction of apoptosis, caspase-3/7 activity was measured using either the Apo-ONE® Homogeneous Caspase-3/7 Assay (black plate) or the Caspase-Glo™ 3/7 Assay (white plate) following the assay protocols (Technical Bulletin #TB295 or Technical Bulletin #TB323, respectively). Briefly, an equal volume of room temperature reagent was added directly to the cell culture plates that had been equilibrated to room temperature. The plates were shaken at 500rpm for 30 seconds and measured for fluorescent or luminescent output at various times following reagent addition (1, 2.5, 5 or 18 hours). Between readings, the plates were stored at room temperature, protected from light. Fluorescence for the Apo-ONE® Homogeneous Caspase-3/7 Assay was measured in a BMG POLARStar fluorescent plate reader with a 480/520 excitation/emission filter and a gain setting of 25. Luminescence for the Caspase-Glo™ 3/7 Assay was measured in a Berthold EG&G MicroLumat Plus luminometer with a 5.0-second read time (RLU factor = 10.0).

## Results and Discussion

We used staurosporine, a general protein kinase inhibitor, to induce apoptosis in the human neuroblastoma cell line SH-SY5Y. Staurosporine treatment is highly effective at inducing apoptosis in this cell type (9,10). Cells were treated with staurosporine or vehicle (DMSO) in the presence or absence of the caspase-3/7 inhibitor, Z-VAD-FMK, which is a cell-permeable caspase inhibitor that irreversibly binds to the catalytic site of caspase proteases and can inhibit the induction of apoptosis.

# Caspase Activity in Neuroblastoma Cells

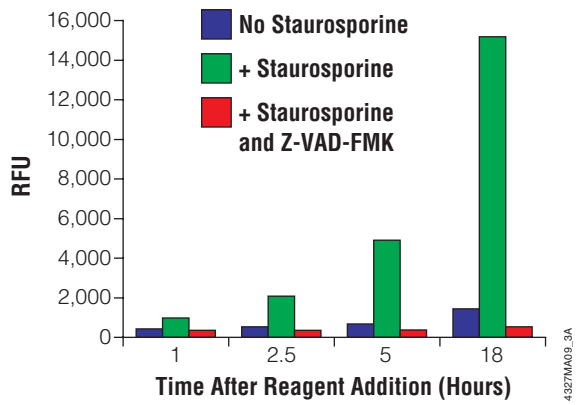


**Figure 1. Detection of caspase-3/7 activity in staurosporine- or vehicle-treated SH-SY5Y cells in the presence or absence of Z-VAD-FMK inhibitor using the Apo-ONE® Homogeneous Caspase-3/7 Assay.** Following reagent addition, the fluorescent signal (RFU) was measured at 1, 2.5, 5, or 18 hours. The results are the average of replicates of 8 wells per treatment.

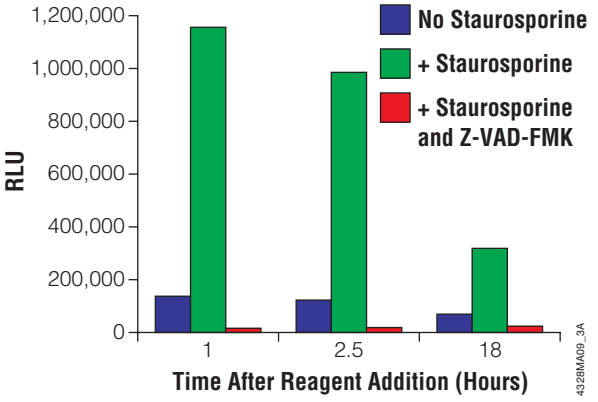


**Figure 2. Detection of caspase-3/7 activity in staurosporine- or vehicle-treated SH-SY5Y cells in the presence or absence of Z-VAD-FMK inhibitor using the Caspase-Glo™ 3/7 Assay.** Following reagent addition, the luminescent signal (RLU) was measured at 1, 2.5 or 18 hours. The results are the average of replicates of 8 wells per treatment.

The results are shown in Figure 1 for the Apo-ONE® Assay and in Figure 2 for the Caspase-Glo™ Assay. Both assay systems effectively detect caspase activity and thus apoptosis induction in staurosporine-treated SH-SY5Y cells.

For the luminescent Caspase-Glo™ Assay, maximum signal is reached when the caspase and luciferase enzymes reach a steady state; in this experiment, maximum signal was reached 1 hour following assay initiation, and slowly decayed over the 18-hour time frame. However, even following the overnight incubation period, the signal from the staurosporine-treated cells was still 15-fold above the vehicle-treated control.For the Apo-ONE® Assay, the fluorescent substrate accumulates over time, producing a signal detectable as early as 1 hour that continues to increase in strength. Maximum sensitivity using the Apo-ONE® Assay was reached at 18 hours.

Both assays showed very low background, and adding the Z-VAD-FMK caspase inhibitor dramatically reduced the fluorescent or luminescent signal to approximately background in the staurosporine-treated cells.

## Summary

Both the Apo-ONE® Homogeneous Caspase-3/7 Assay and the Caspase-Glo™ 3/7 Assay provide easy and sensitive methods for measuring caspase-3/7 activity, and thus apoptosis induction. These apoptosis assays have been used in a wide variety of cell types and systems in addition to the human neuroblastoma cell line used here (7, 8, 11, 12). ■

## Online Tool

See the Apoptosis Assistant online at **www.promega.com/apoasst/**

# Caspase Activity in Neuroblastoma Cells

## References


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## Protocols

*Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin* #TB295
(www.promega.com/tbs/tb295/tb295.html)

*Caspase-Glo™ 3/7 Assay Technical Bulletin* #TB323
(www.promega.com/tbs/tb323/tb323.html)

*Automated Caspase-Glo™ 3/7 Assay Protocol* #EP017
(www.promega.com/tbs/ep017/ep017.html)

*Automated Apo-ONE® Homogeneous Caspase-3/7 Assay Protocol* #EP012
(www.promega.com/tbs/ep012/ep012.html)

## Ordering Information

| Product | Size | Cat.# |
|---|---|---|
| Apo-ONE® Homogeneous Caspase-3/7 Assay[a] | 1ml | G7792 |
| | 10ml | G7790 |
| | 100ml | G7791 |
| Caspase-Glo™ 3/7 Assay[b,c] | 2.5ml | G8090 |
| | 10ml | G8091 |
| | 100ml | G8092 |
| Caspase Inhibitor Z-VAD-FMK | 50µl | G7231 |
| | 125µl | G7232 |

[a]This product is covered by U.S. Pat. Nos. 4,557,862 and 4,640,893 and is sold for research use only. All other uses, including but not limited to use as a clinical diagnostic or therapeutic, require a separate license. Please contact Promega Corporation for details relating to obtaining a license for such other use.

[b]Patent Pending.

[c]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.