CITATION NOTE: DEVELOPMENT OF A HOMOGENEOUS BIOLUMINESCENT CASPASE ASSAY

REVIEW BY: KARI KENEFICK, M.S., PROMEGA CORPORATION

Review of:
O'Brien, M.A. *et al.* (2005) Homogeneous, bioluminescent protease assays: caspase-3 as a model. *J. Biomol. Screen.* **10**, 137–48.

Introduction

As mediators of cellular apoptosis (programmed cell death) caspases are molecules of interest as potential targets in drug development. Caspase-3 is particularly important due to its recognition as a "key executioner caspase"; caspase-3 activation indicates progression of the apoptotic path to an irreversible stage. Research interests lie in both caspase inhibition and induction: the ability to inhibit caspase could potentially block an apoptotic pathway in cells, while the ability to induce caspase, and thus apoptosis has potential use in cancer therapy.

To make the search for new drugs more economically viable, biopharmaceutical companies use high-throughput screening (HTS). Using multiwell plates with automated reagent handling systems, thousands of compounds can be tested simultaneously. Rapid, sensitive, homogeneous assays where reaction reagents are added in a single automated step are essential to HTS. Traditional fluorescent homogeneous assays for caspase suffer limited sensitivity due to increased background from residual fluorescence of peptide-conjugated fluorophores and spectral overlap of peptide-conjugated substrates and their fluorescent products (1,2).

Developing a One-Step Luminescent Assay for Caspase Activity

In this report the researchers developed a one-step homogeneous luminescent assay for caspase. They first synthesized a peptide-conjugated aminoluciferin, Z-DEVD-aminoluciferin, as the luminescent substrate, then combined the substrate with a stable recombinant firefly luciferase, Ultra-Glo™ Luciferase (Promega Corporation, reference 3). The homogeneous luminescent assay was optimized for assays containing recombinant enzyme and for cell-based assays in 96- and 384-well plates.

Finally, the homogeneous luminescent caspase assay was compared to homogeneous fluorescent assays currently available. Signal-to-noise ratios and Z´ factor values were used to compare assay sensitivity and applicability to HTS systems, respectively. Figure 1 compares the luminescent assay using Z-DEVD-aminoluciferin with two assays that use fluorescent substrates. The assays were run in parallel and results were read with a luminometer or fluorometers at various intervals. Signal-to-noise ratios (S/N) were plotted for one and three hours after substrate addition. Panel A shows data for titration of caspase enzyme, while Panel B shows anti-Fas treated Jurkat cells.

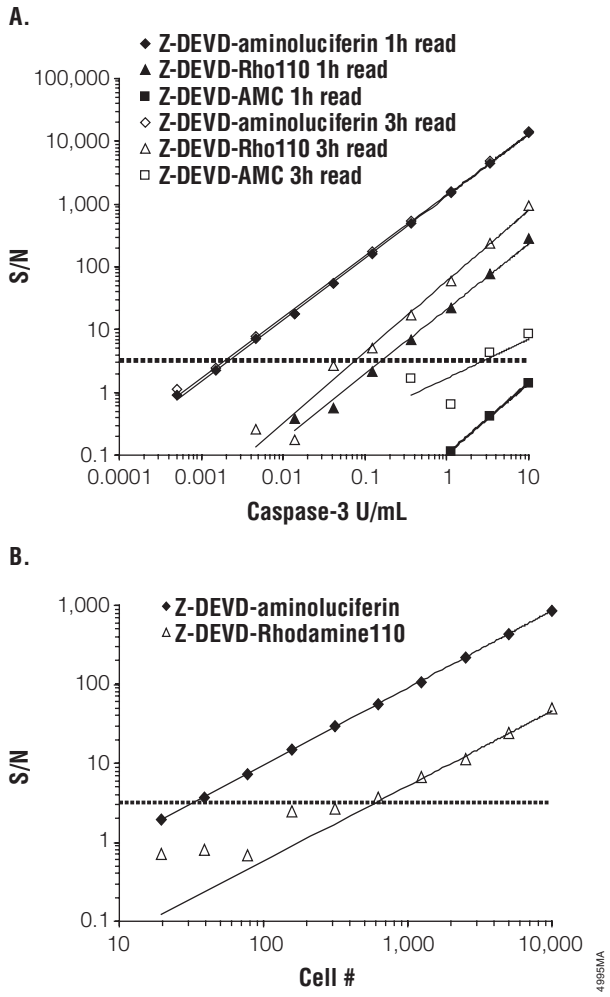

Figure 1. A comparison between bioluminescent and fluorescent caspase assays. Reprinted with permission of the *Journal of Biomolecular Screening* (SAGE publications).

In Panel A, the signal-to-noise ratios for Z-DEVD-aminoluciferin at one hour were approximately 80-fold greater than those for the $(Z\text{-DEVD})_2$-R110 and >1,000-fold higher than for the Z-DEVD-AMC fluorescent substrate.

At three hours the S/N ratio differences were less dramatic, with a 60-fold higher reading for the luminescent substrate over $(Z\text{-}DEVD)_2$-R110 and about 1,000-fold higher than the other fluorescent substrate.

For Panel B, bioluminescent and fluorescent assays were run in parallel using the same anti-Fas treated Jurkat cells. Background levels were determined from wells containing medium but no cells. The S/N ratios one hour after substrate addition were 20-fold higher for the luminescent assay than for the $(Z\text{-}DEVD)_2$-R110 fluorescent assay. The limit of detection for the bioluminescent assay was approximately 30 induced cells, while the fluorescent assay had a limit of detection of approximately 600 induced cells.

Review of the caspase titration data as well as induced caspase detection in anti-Fas treated Jurkat cells shows that this homogeneous bioluminescent assay has better sensitivity and lower limits of detection than the comparable one-step fluorescent assays for caspase. Z´ factor data showed that the homogeneous bioluminescent assay provides suitable Z´ factor values for use in HTS. ■

References

1. Leytus, S.P., Mehado, L.L. and Mangel, W.P. (1983) *Biochem. J.* **209,** 299–307.
2. Liu, J. *et al.* (1999) *Bioorg. Med. Chem. Lett.* **9**, 3231–6.
3. O'Brien, M. *et al.* (2005) *J. Biomol. Screen.* **10**, 137–48.

Protocol

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

Ordering Information

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	2.5ml	G8090
	10ml	G8091
	100ml	G8092

For Laboratory Use.

[a]U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 and other patents pending.

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

Caspase-Glo is a registered trademark of Promega Corporation. Ultra-Glo is a trademark of Promega Corporation.