USING CASPASE-GLO® ASSAYS TO PERFORM WHOLE ORGANISM SCREENING FOR APOPTOSIS IN ZEBRAFISH EMBRYOS

FROM GEIGER, G.A. *ET AL*. (2006) ZEBRAFISH AS A "BIOSENSOR"? EFFECTS OF IONIZING RADIATION AND AMIFOSTINE ON EMBRYONIC VIABILITY AND DEVELOPMENT. *CANCER RES*. **66**, 8172–81.

REVIEW BY MICHELE ARDUENGO, PROMEGA CORPORATION

The authors of this paper investigate disruption of embryonic development and viability in zebrafish. In this study, they observe many of the predicted morphological effects of radiation treatment including retinal and optic atrophy, lens opacification and microencephaly. These abnormalities also show age- and dosage-dependence.

In this study, the authors evaluate caspase activity as an indirect indicator of radiation effects. The caspase family of cysteine proteases are the central mediators of the apoptosis proteolytic cascade leading to cell death and elimination of compromised cells. They use the Caspase-Glo® 8 and 9 Assays[a–c] (Cat.# G8200 and G8210) to investigate caspase activity in response to radiation exposure alone or radiation exposure in the presence of the radiomodifier and chemoprotectant, amifostine. The Caspase-Glo® Assays are rapid and require minimal sample preparation, making them ideal for screening applications.

Briefly, embryos were grown in densities of 20 or less in 5ml of growth medium and exposed to radiation at four hours post fertilization (hpf). Just prior to 6hpf, embryos were plated onto 96-well black plates with clear bottoms at a density of 10 embryos per well. Caspase-Glo® Reagents were added, and plates were read after two hours using a Kodak 4000MM Imaging System. The authors examined caspase activation after irradiation, mock irradiation, or irradiation in the presence of amifostine. Individual components of the assay (buffer and substrate) were tested as well as the complete assay in the absence of embryos.

The results show that the Caspase-Glo® Assays reliably indicate caspase-8 and caspase-9 activity in whole zebrafish embryos (Figure 1). No activity was observed when individual components of the assay were used or in the no-embryo control. Activity increased with increasing numbers of embryos or increased irradiation. Radiation-dose-dependent activity of caspase-8 and caspase-9 was clearly demonstrated as well. Caspase-9 activity was reduced in the presence of the pan-caspase inhibitor Z-VAD-FMK, and amifostine also reduced caspase-9 activity.

This work shows that the Caspase-Glo® Assays can be used as a tool to screen for apoptosis in whole zebrafish embryos, providing a sensitive vertebrate model system for screening drug and other treatment effects. The assays are fast and simple, requiring minimal intervention by the scientist, allowing high-throughput application, and providing highly reproducible results.

Ordering Information

Product	Size	Cat.#
Caspase-Glo® 8 Assay	2.5ml	G8200
	10ml	G8201
	100ml	G8202
Caspase-Glo® 9 Assay	2.5ml	G8210
	10ml	G8211
	100ml	G8212

For Laboratory Use.

[a]U.S. Pat. No. 7,148,030 and other patents pending.

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

Are you interested in learning more about apoptosis and available reagents for studying apoptotic events?

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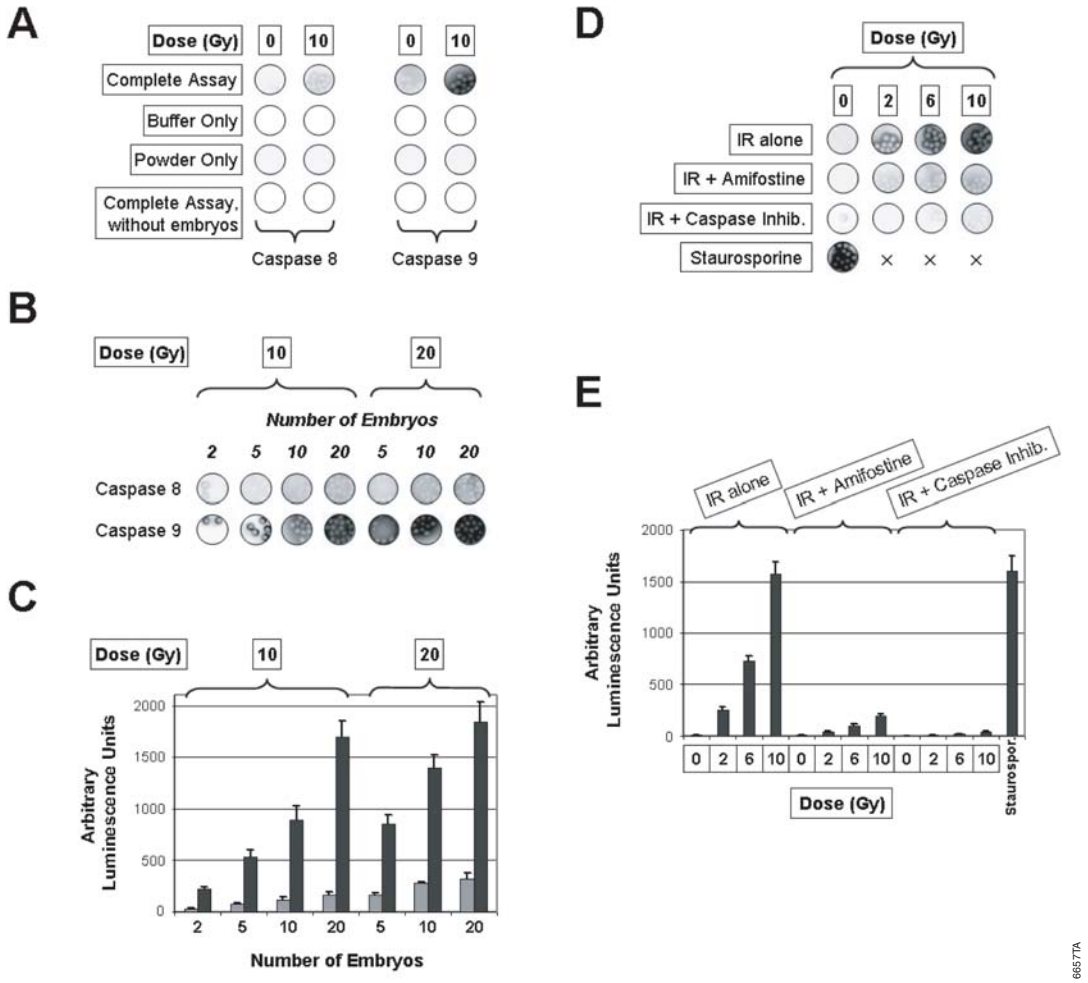

Figure 1. Bioluminescent imaging of caspase activation in zebrafish embryos. Panel A. Zebrafish embryos (10/well) were mock irradiated (0 Gray) or irradiated with 10 Gray (Gy) at 4hpf. Ten embryos from the respective treatment groups were immediately placed into individual wells of a 96-well microplate, followed by a 30-minute exposure under gentle agitation to either the combined components of the LETD-aminoluciferin (caspase-8) or the LEHD-aminoluciferin (caspase-9) assay (Complete assay); lysis buffer only (Buffer Only); reagent reconstituted without lysis buffer (Powder Only); or the combined components of the respective caspase-8 and caspase-9 assay reagents in the absence of embryos (Complete Assay, without embryos). The LETD- or LEHD-aminoluciferin powder was reconstituted in E3 embryo medium. Imaging was done 1 hour after mock irradiation or irradiation. For maximal clarity and contrast, the embryo-containing wells are shown as negative images (i.e., emission of light results in darker images). **Panel B.** Zebrafish embryos were irradiated with 10 or 20Gy, and the indicated numbers of embryos from either treatment group were placed into individual wells of a 96-well microplate. All embryos were then assayed with the complete components of the caspase-8 and caspase-9 assays at 1 hour after irradiation. **Panel C.** Histograms showing the relative levels of emitted signal from the experiment shown in Panel B, displayed in arbitrary units of luminescence. Black bars, caspase-9; hatched bars, caspase-8. **Panel D.** Zebrafish embryos (10/well) were mock irradiated (0Gy) or irradiated with 2, 6 or 10Gy at 4hpf in medium only (IR alone), in the presence of amifostine (IR + amifostine), in the presence of a pan-caspase inhibitor (IR + Caspase Inhib.), or mock irradiated but exposed to staurosporine (Staurosporine). Ten embryos from each respective treatment group were immediately placed into individual wells of a 96-well plate, followed by a 30-minute exposure under gentle agitation to the combined components of the caspase-9 assay. Imaging was done at 1 hour after mock irradiation or irradiation. **Panel E.** Histograms showing the relative levels of emitted signal from the experiment shown in Panel D, displayed in arbitrary units of luminescence. All wells are shown as negative images. Figure reprinted with the kind permission of G. Kao and the American Association of Cancer Research from Geiger *et al.* (2006) *Cancer Res.* **66**, 8172–81.