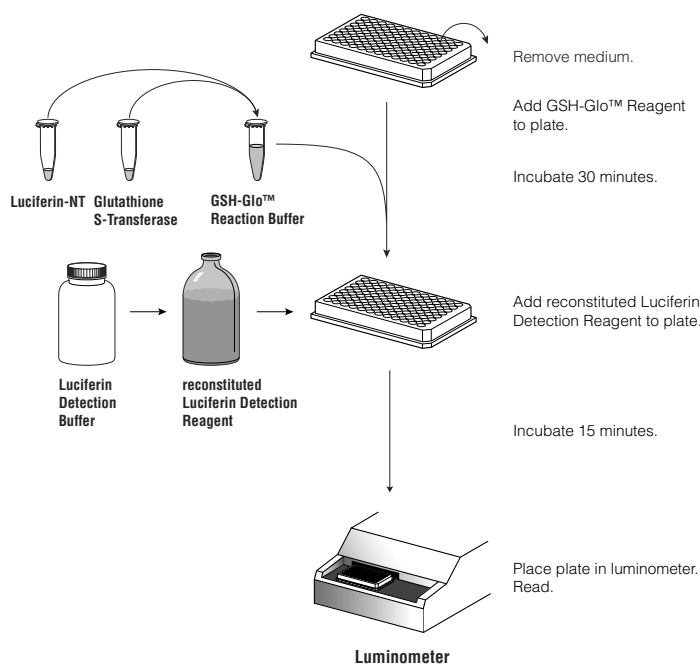


## GSH-Glo™ Glutathione Assay

### Description

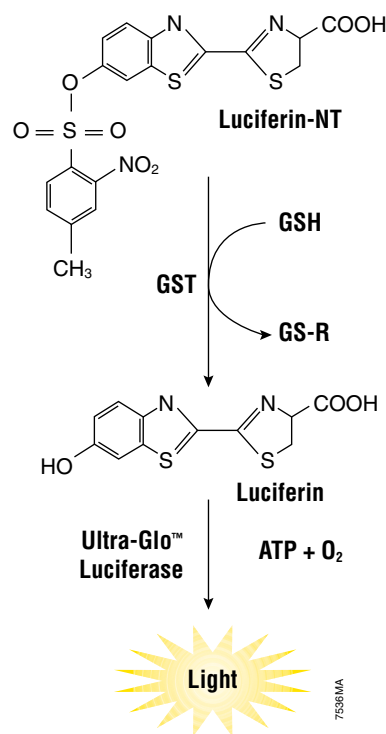
The GSH-Glo™ Glutathione Assay is a luminescent-based assay for the detection and quantification of glutathione (GSH) in cells or in various biological samples. A change in GSH levels is important in assessment of toxicological responses and is an indicator of oxidative stress, potentially leading to apoptosis or cell death. The assay is based on the conversion of a luciferin derivative into luciferin in the presence of GSH (figure 2). The reaction is catalyzed by a glutathione S-transferase (GST) enzyme supplied in the kit. The luciferin formed is detected in a coupled reaction using Ultra-Glo™ Recombinant Luciferase that generates a glow-type luminescence that is proportional to the amount of glutathione present in cells.



**Figure 1.** The steps involved in the GSH-Glo™ Glutathione Assay. Medium is removed from the plate containing samples, and the prepared GSH-Glo™ Reaction Buffer is then added to the plate. After a 30-minute incubation, reconstituted Luciferin Detection Reagent is added to the plate. Following a 15-minute incubation, the plate is read in a luminometer.

The assay provides a simple, fast and sensitive alternative to colorimetric and fluorescent methods and can be easily adapted to high-throughput applications. The assay can also be adapted for measuring total glutathione levels.

- **Versatile:** Measure reduced glutathione and/or total glutathione levels from cells, tissue or blood.
- **Fast and Easy to Use:** Measure GSH levels directly in cell culture wells; no deproteinization step required.
- **Excellent Sensitivity:** Measure GSH levels from as few as several hundred cells. Easily scalable to 384-well plates.
- **Robust:** No interference by oxidized GSH or reducing agents.
- **Proven Luminescent Technology:** Powered by Ultra-Glo™ Luciferase. No interference by fluorescent compounds.

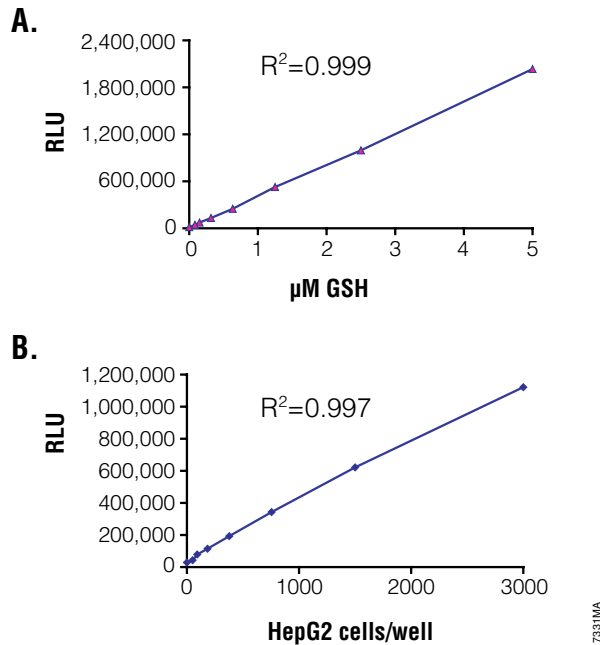


6877MA

7536MA

**Figure 2.** Schematic of the GSH-Glo™ Glutathione Assay. The assay is performed in two steps. In the first step, cells are lysed in the presence of the luciferin-NT substrate and glutathione S-transferase (GST). Glutathione in the cells drives the formation of luciferin. In the second step, Luciferin Detection Reagent is added to produce light that is directly proportional to the amount of GSH in the reaction.





**Figure 3. Titration data using the GSH-Glo™ Glutathione Assay. Panel A.** A standard curve was generated by serial twofold dilutions of a glutathione 10X solution in the wells of a 384-well plate. **Panel B.** Serial twofold dilution of HepG2 cells were plated and allowed to attach overnight. The cells were assayed using the protocol outlined in Promega Technical Bulletin #TB369.

### Ordering Information

Product	Size	Cat.#
GSH-Glo™ Glutathione Assay	10ml	V6911
	50ml	V6912

10ml size contains sufficient reagent for 100 assays of 100μl each in 96-well plates or 400 assays of 25μl in 384-well plates.

50ml size contains sufficient reagent for 500 assays of 100μl each in 96-well plates of 2,000 assays or 25μl each in 384-well plates.

#### Each System Includes:

- Luciferin-NT Substrate
- GSH-Glo™ Reaction Buffer
- Glutathione S-transferase
- Glutathione, 5mM
- Luciferin Detection reagent
- Luciferin Detection Buffer

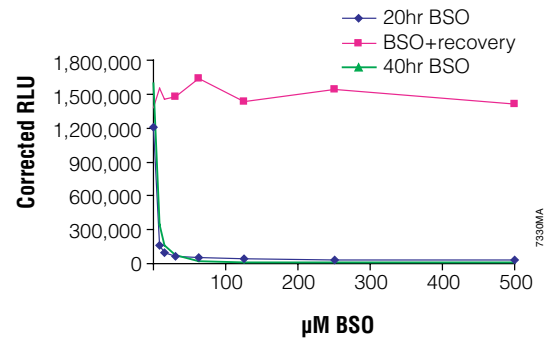
Certain applications of this product may require licenses from others.

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.

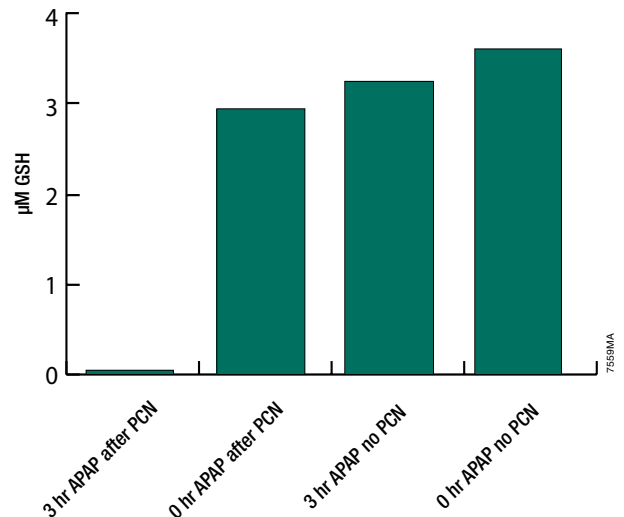
Patent Pending.

Products may be covered by pending or issued patents.

Please visit [www.promega.com](http://www.promega.com) for more information.



**Figure 4. Measurement of GSH Depletion and Recovery in Treated Cells.** HeLa cells (5000 cells/well in 96-well format) were exposed to L-Buthionine-sulfoximine (BSO). BSO inhibits GSH synthesis thus reducing cellular GSH levels. **20hr BSO-** cells were treated with BSO for 20 hours and assayed for GSH. **40hrs BSO-** cells treated with BSO, washed at 20 hours and fresh media plus BSO added. Cells were assayed for GSH after 40 hours. **BSO+Recovery-** cells treated with BSO for 20 hours. After 20 hours cells were washed 2X with PBS and covered with fresh media without BSO. Cells assayed for GSH after 40 hours. All samples were assayed for GSH using the GSH-Glo™ Glutathione Assay according to the protocol outlined in Promega Technical Bulletin #TB369.



**Figure 5. GSH depletion by acetaminophen (APAP) in rat hepatocytes.** GSH levels in lysates from adherent cells in 24-well plates were measured with the GSH-Glo Glutathione Assay. GSH concentrations were determined by interpolation from a GSH standard curve generated using the bioluminescent system. Exposure to 5mM acetaminophen for three hours substantially reduced GSH only after treatment for two days with a P450 inducer, 30μM pregnenolone 16α-carbonitrile (PCN).

