TECHNICAL MANUAL

GSH-Glo™ Glutathione Assay

Instructions for Use of Products
V6911 and V6912

Revised 3/15
TB369

GSH-Glo™ Glutathione Assay

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Glutathione (GSH), a three-amino-acid peptide, is an antioxidant found in eukaryotic cells (1–3). Reactive chemical species can cause a drop in GSH levels either by oxidation or reaction with the thiol group. A change in GSH levels is important for assessing toxicological responses and can promote oxidative stress, potentially leading to apoptosis and cell death (4).

The GSH-Glo™ Glutathione Assay[a,b] is a luminescence-based assay for detecting and quantifying glutathione (GSH). The assay is based on the conversion of a luciferin derivative into luciferin in the presence of glutathione, catalyzed by glutathione S-transferase (GST). The signal generated in a coupled reaction with firefly luciferase is proportional to the amount of glutathione present in the sample. Luminescence is generated in less than one hour. The assay (Figure 1) generates a stable luminescent signal and is simple, fast and easily adaptable to multiwell formats such as 96- and 384-well plates. Mammalian cells are grown, treated, lysed and assayed for GSH changes in the same plate. This assay can be used to detect and quantify GSH in cultured cells or in various biological samples.

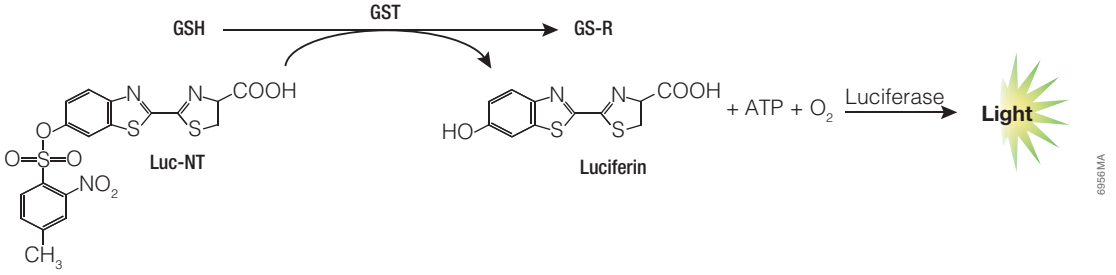

Figure 1. An overview of the GSH-Glo™ Glutathione Assay.

The GSH-Glo™ Glutathione Assay is ideal for many applications, including:

- Measuring glutathione levels in various cell or tissue extracts as an indicator of cell viability or oxidative stress
- Screening drugs and new chemical entities for their capacity to modulate glutathione levels in cells, tissues or blood

Advantages of the GSH-Glo™ Glutathione Assay include:

Speed: The luminescence format eliminates the need for time-consuming analysis such as HPLC. There is no need to deproteinate samples, and centrifugation is not needed.

Signal Stability: Glow-type luminescence provides a stable signal with a half-life greater than 2 hours, eliminating the need for strictly timed luminescent detection.

Greater Sensitivity: Less sample is required in these assays than in typical HPLC or fluorometric methods due to the enhanced sensitivity.

No Fluorescence Interference: Using luminescence to monitor GSH levels eliminates the interference between fluorescent excitation wavelengths and emission wavelengths of reagents and test compounds sometimes seen in fluorescence assays. Such overlap can confound analysis and present misleading or irrelevant data.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GSH-Glo™ Glutathione Assay	**10ml**	**V6911**

Each system contains sufficient reagents for 100 assays of 100µl each in a 96-well plate or 400 assays of 25µl each in 384-well plates. Includes:

- 100µl Luciferin-NT
- 10ml GSH-Glo™ Reaction Buffer
- 100µl Glutathione S-Transferase
- 100µl Glutathione, 5mM
- 1 bottle Luciferin Detection Reagent
- 10ml Reconstitution Buffer with esterase

PRODUCT	SIZE	CAT.#
GSH-Glo™ Glutathione Assay	**50ml**	**V6912**

Each system contains sufficient reagents for 500 assays of 100µl each in 96-well plates or 2,000 assays of 25µl each in 384-well plates. Includes:

- 500µl Luciferin-NT
- 50ml GSH-Glo™ Reaction Buffer
- 500µl Glutathione S-Transferase
- 100µl Glutathione, 5mM
- 1 bottle Luciferin Detection Reagent
- 50ml Reconstitution Buffer with esterase

Storage Conditions: Store all components at –20°C protected from light. The reconstituted Luciferin Detection Reagent can be stored at room temperature (approximately 22°C) for 1 week or –20°C for 2 months with no change in activity. The activity of the reconstituted Luciferin Detection Reagent decreased approximately 10% when the reagent was stored at 4°C for 2 months. **Prepare the GSH-Glo™ Reagent immediately prior to use. Do not store the GSH-Glo™ Reagent for future use.**

3. Performing the GSH-Glo™ Glutathione Assay

3.A. General Considerations

Detection of GSH using the GSH-Glo™ Glutathione Assay is the result of combining two chemical reactions. The chemical reactions are outlined in Figure 1 and in a schematic overview of the assay, shown in Figure 2.

1. The GSH-Glo™ Reaction. The first reaction involves generation of luciferin from a luminogenic substrate, which is catalyzed by GST in the presence of glutathione. The recommended substrate concentration is the apparent K_m of the GST enzyme, which results in a strong signal that varies depending on the concentration of glutathione in the sample. The assay is compatible with cells, tissue or blood samples.
2. The Luciferin Detection Reaction. The luciferin produced in Step 1 of the GSH-Glo™ Assay is detected as a luminescent signal generated by the luciferase enzyme. Step 2 is initiated by adding an equal volume of Luciferin Detection Reagent, which simultaneously stops the GSH-Glo™ Reaction and initiates a luminescent signal that is directly proportional to the amount of luciferin formed in Step 1. The GSH-Glo™ Assay uses a proprietary stabilized luciferase (Ultra-Glo™ Luciferase) to produce reaction conditions that generate a stable "glow-type" luminescent signal. The half-life of the luminescent signal from the GSH-Glo™ Assay is greater than 2 hours, eliminating the need for strictly timed luminescence detection.

Tips for Success:

- Include a control on each plate. Comparison to the control allows correction of small variations in luminescence that can occur over time or due to other variables such as changes in temperature.
- Because 15 minutes is required for the luminescence to stabilize, add the Luciferin Detection Reagent to the plates before putting them into the luminometer. The reagent is not designed for use with the automated reagent injectors that are integrated into some luminometers.
- To achieve linear assay performance at low light levels, subtract background luminescence from all readings. Some instruments also require verification of linear response at high light levels (consult the instrument manual).
- Use multiwell plates (or tubes, Section 5.D) that are compatible with your luminometer. Consult the luminometer instructions for proper use of the instrument.
- Use an integration time of 0.25–1 second per well as a luminometer guideline. Relative light units are arbitrary units that vary depending on the instrument manufacturer and model. Absolute readings from one luminometer may not match those from another model.

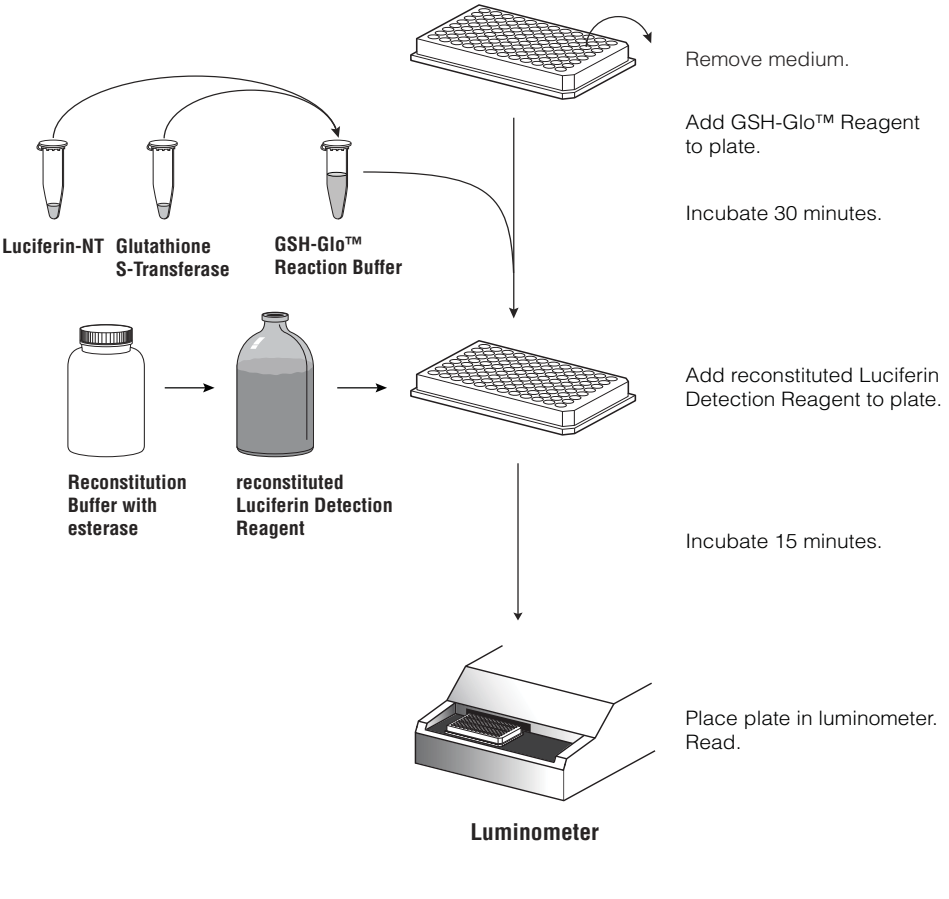

Figure 2. The steps involved in the GSH-Glo™ Glutathione Assay. Medium is removed from the plate containing samples, and Luciferin-NT and Glutathione S-Transferase are added to GSH-Glo™ Reaction Buffer to make GSH-Glo™ Reagent, which is then added to the plate. After a 30-minute incubation, reconstituted Luciferin Detection Reagent is added to the plate. Following a 15-minute incubation, luminescence is measured using a luminometer.

3.B. Reagent Preparation

Materials to Be Supplied by the User
(Solution compositions are provided in Section 5.A.)

- distilled or deionized water
- PBS
- PBS containing 2mM EDTA
- PBS containing heparin
- blood collection tubes with anticoagulant
- white, opaque polystrene flat-bottom 96- or 384-well plates
- luminometer capable of reading multiwell plates
- multichannel pipette or automated repeating pipettor
- tissue homogenizer

GSH-Glo™ Reagent 1X: Use with adherent mammalian cells. The GSH-Glo™ Reagent contains Luciferin-NT substrate and Glutathione S-Transferase diluted 1:100 in GSH-Glo™ Reaction Buffer. Each reaction (well) of a 96-well plate requires 100µl of GSH-Glo™ Reagent; adjust the total volume prepared for the number of assay wells. A complete 96-well plate requires 10ml of GSH-Glo™ Reagent, which is prepared by adding 100µl of Luciferin-NT substrate and 100µl of Glutathione S-Transferase to 10ml of GSH-Glo™ Reaction Buffer. Prepare GSH-Glo™ Reagent 1X immediately prior to use. Do not store prepared reagent for future use.

GSH-Glo™ Reagent 2X: Use with suspension mammalian cells and tissue extracts. The GSH-Glo™ Reagent 2X contains Luciferin-NT substrate and Glutathione S-Transferase diluted 1:50 in GSH-Glo™ Reaction Buffer. Each reaction (well) of a 96-well plate requires 50µl of GSH-Glo™ Reagent 2X; adjust the total volume of GSH-Glo™ Reagent 2X prepared for the number of assay wells. Add 50µl per well of suspension cells or tissue extracts. The total volume in each well is 100µl. A complete 96-well plate requires 5ml of GSH-Glo™ Reagent 2X, prepared by adding 100µl of Luciferin-NT substrate and 100µl of Glutathione S-Transferase to 5ml of GSH-Glo™ Reaction Buffer. Prepare GSH-Glo™ Reagent 2X immediately prior to use. Do not store prepared reagent for future use.

GSH Standard Curve: Prepare a standard curve using a GSH standard solution (Glutathione, 5mM) to facilitate conversion of luminescence (in RLU) to GSH concentration. Dilute the Glutathione, 5mM stock (1:100) in water, and then perform serial 1:1 dilutions by combining an equal volume of Glutathione and an equal volume of water. Transfer 10µl of each diluted standard to the appropriate wells for the assay. The final concentration of Glutathione, 5mM, will range from 0µM to 5µM. Figure 3 shows a standard curve.

Luciferin Detection Reagent: Transfer the contents of one bottle of Reconstitution Buffer with esterase to the amber bottle of lyophilized Luciferin Detection Reagent. Mix by inversion until the substrate is thoroughly dissolved. Do not vortex.

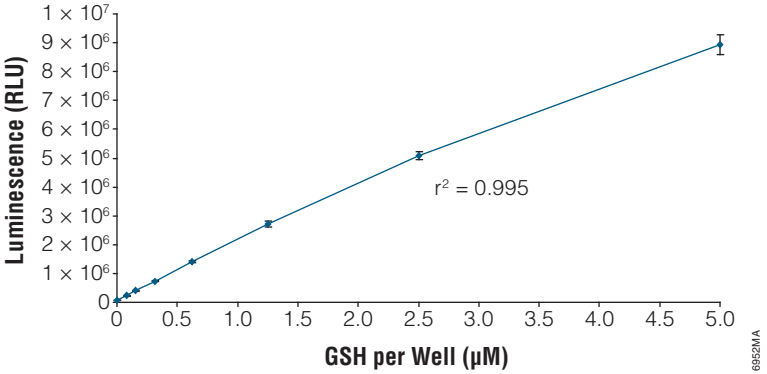

Figure 3. Glutathione standard curve. A standard curve was generated by serial twofold dilutions of a 10X glutathione solution. Ten microliters of the solution was added to triplicate wells of a 96-well plate. GSH-Glo™ Reagent (100µl) was added to each well, and the plate was incubated for 30 minutes. An equal volume (100µl) of reconstituted Luciferin Detection Reagent was added to each well, and after 15 minutes of incubation, luminescence was measured using a luminometer.

Notes:

1. Luciferin-NT substrate, GSH-Glo™ Reaction Buffer and Glutathione S-Transferase are stable at room temperature for several hours as separate solutions. Do not thaw the solutions above 25°C, and mix well after thawing. The most convenient and effective method for thawing is to place the reagent in a room-temperature water bath.
2. Light intensity is a measure of the rate of catalysis by the luciferase and is temperature-dependent. The temperature optimum for firefly luciferase activity is room temperature (20–25°C), so reagents should be equilibrated to room temperature before measurements begin.

3.C. Assay Procedure for Adherent Mammalian Cells

Note: Most mammalian cell culture media contain serum and phenol red, which can interfere with the GSH-Glo™ Assay chemistry. For additional information see the Appendix, Section 5.E.

1. Remove multiwell plates containing adherent mammalian cells with and without test compounds from the incubator. Generally 1,000–10,000 cells/well are used for a 96-well plate, or 300–3,000 cells/well are used for a 384-well plate.
2. Carefully remove the culture medium from the wells.
3. Add 100µl of prepared 1X GSH-Glo™ Reagent to each well of a 96-well plate (for 384-well plates, add 25µl per well). Mix briefly on a plate shaker. Incubate at room temperature for 30 minutes.
4. Add 100µl of reconstituted Luciferin Detection Reagent to each well of a 96-well plate (for 384-well plates, add 25µl per well). Mix briefly on a plate shaker. Incubate for 15 minutes.
5. Measure luminescence.

3.D. Assay Procedure for Mammalian Cells in Suspension

Note: Most mammalian cell culture media contains serum and phenol red, which interfere with the GSH-Glo™ Assay chemistry; therefore, suspension cells must be assayed in PBS.

1. Harvest suspension cells by centrifugation, and dilute in PBS. Dispense cells at 50µl per well into 96-well plates (15µl per well into 384-well plates). Generally 1,000–10,000 cells/well are sufficient for measurement in 96-well plates, and 300–3,000 cells/well are sufficient for measurement in 384-well plates.
2. For 96-well plates, add 50µl per well of prepared GSH-Glo™ Reagent 2X (for 384-well plates, add 15µl per well). Mix briefly on a plate shaker. Incubate at room temperature for 30 minutes.
3. Add 100µl per well of prepared Luciferin Detection Reagent to a 96-well plate (for 384-well plates, add 30µl per well). Mix briefly on a plate shaker. Incubate for 15 minutes.
4. Measure luminescence.

3.E. Assay Procedure for Tissue Extracts

1. Perfuse tissue with PBS containing heparin to remove blood and clots. Weigh tissue. The GSH-Glo™ Glutathione Assay is quite sensitive, so only a small amount of tissue is necessary (e.g., <10mg).
2. Homogenize tissue in PBS containing 2mM EDTA. Use 1–2ml of PBS/EDTA per 10mg of tissue. Centrifuge the extract, and collect the supernatant. Place the supernatant on ice, and assay directly or store at –70ºC if extracts are not assayed the same day.
3. Add 50µl of tissue extract to each well of a 96-well plate. (Extracts may require additional dilution in PBS/EDTA.)
4. Add 50µl of prepared GSH-Glo™ Reagent 2X to each well of a 96-well plate. Incubate at room temperature for 30 minutes.
5. Add 100µl of reconstituted Luciferin Detection Reagent to each well of a 96-well plate. Mix briefly on a plate shaker. Incubate for 15 minutes.
6. Measure luminescence.

3.F. Assay Procedure for Whole Blood and Erythrocyte Lysate

Note: Serum and plasma samples contain levels of glutathione that are below the level of detection of this assay.

1. Collect blood using an anticoagulant such as heparin, citrate or EDTA.
2. Prepare a lysate:
   a. Whole blood lysate: Mix blood 8–10 times with anticoagulant by gently inverting the tube. Dilute blood 1:5 in GSH-Glo™ Reaction Buffer. Incubate on ice for 15 minutes to lyse. Centrifuge at 10,000 × *g* in a microcentrifuge for 15 minutes at 4°C. Collect the supernatant (lysed whole blood sample). Store on ice for immediate measurement or at –20°C for several months.
   b. Erythrocyte lysate: Mix blood 8–10 times with anticoagulant by gently inverting the tube. Centrifuge at 1,000 × *g* for 15 minutes at 4°C. Carefully remove the top yellow plasma layer and white buffy coat layer, and discard (see the note at the top of this page). Dilute erythrocytes (cell pellet) 1:5 in GSH-Glo™ Reaction Buffer, and mix by inversion to aid lysis. Centrifuge at 10,000 × *g* in a microcentrifuge for 15 minutes at 4°C. Collect the supernatant (erythrocyte lysate sample). Store on ice for immediate measurement or at –20°C for several months.
3. Both whole blood and erythrocyte lysates require additional dilution prior to measurement. Dilute lysates 1:15 to 1:50 in deionized water.
4. Transfer 10µl of diluted lysate to multiple wells of a 96-well plate. Add 100µl of 1X GSH-Glo™ Reagent. Mix briefly on a plate shaker. Incubate at room temperature for 30 minutes.
5. Add 100µl of prepared Luciferin Detection Reagent to each well of a 96-well plate. Mix briefly on a plate shaker. Incubate for 15 minutes.
6. Measure luminescence.

4. Analysis of Results

Calculate the net GSH-dependent luminescence (net RLU) by subtracting the average luminescence of the negative control reactions from that of GSH-containing reactions. The net signal from GSH reactions in the absence of test compound represents the total GSH activity. Changes from the average net signal for total GSH activity to the net signals for reactions with test compound reflect the effect of the compound on GSH levels. Changes in luminescent signal will typically be seen as a decrease because reactive chemical species and unintended drug interactions often cause a drop in GSH levels either by oxidation or reaction with the thiol group.

To measure total GSH (reduced GSH plus oxidized GSSG), a reducing agent such as TCEP can be added to test wells. TCEP used at 500µM–1mM does not interfere significantly with the GSH-Glo™ Glutathione Assay and will reduce any oxidized glutathione present in the samples.

5. Appendix

5.A. Composition of Buffers and Solutions

GSH-Glo™ Reaction Buffer

50mM Tricine (pH 7.9)

PBS, 1X

137mM NaCl
2.68mM KCl
1.47mM KH_2PO_4
8.1mM Na_2HPO_4

5.B. References

1. Sies, H. (1999) Glutathione and its role in cellular functions. *Free Radic. Biol. Med.* **27**, 916–21.
2. Griffith, O.W. (1999) Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic. Biol. Med.* **27**, 922–35.
3. Pompella A. *et al.* (2003) The changing faces of glutathione, a cellular protagonist. *Biochem. Pharmacol.* **66**, 1499–1503.
4. Townsend, D.M., Tew, K.D. and Tapiero, H. (2003) The importance of glutathione in human disease. *Biomed. Pharm.* **57**, 145–55.
5. Griffith, O.W. and Meister, A. (1985) Origin and turnover of mitochondrial glutathione. *Proc. Natl. Acad. Sci. USA* **82**, 4668–72.

5.C. Sample Data

Representative data are shown in Figures 4–6.

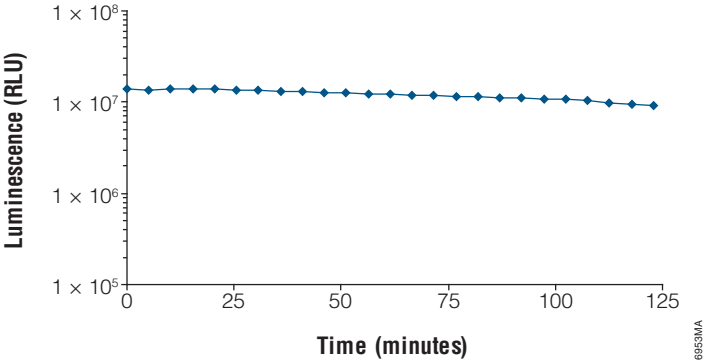

Figure 4. Steady state luminescence. Glutathione (5µM) was added to 100µl of GSH-Glo™ Reagent and incubated for 30 minutes. The reaction was terminated by adding 100µl of Luciferin Detection Reagent and monitored over time using the GloMax® 96 Microplate Luminometer (Cat.# E6501). The half-life under assay conditions was calculated to be greater than 2 hours.

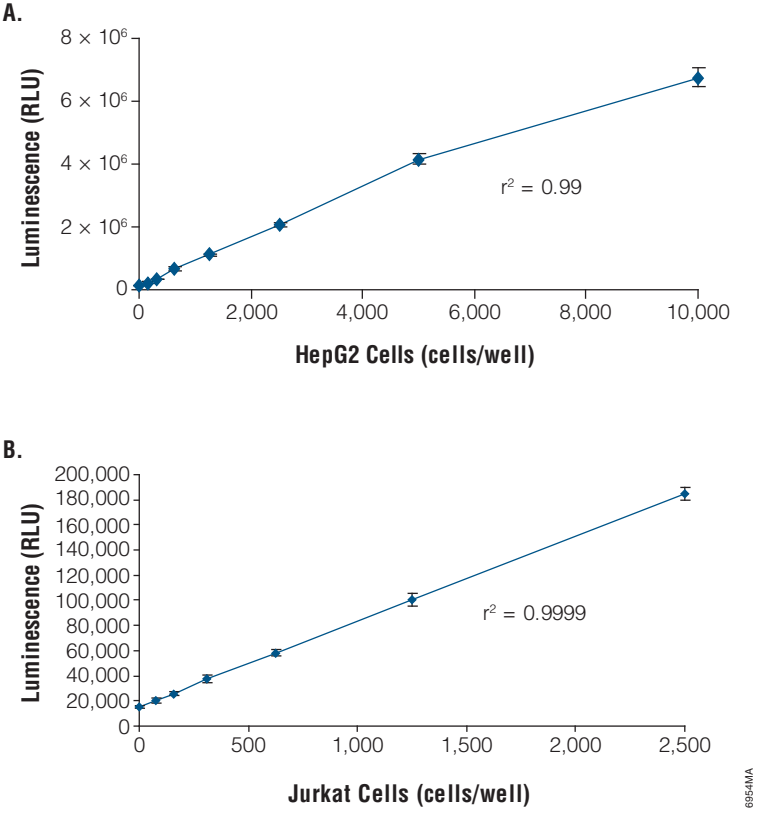

Figure 5. Cell titration. The GSH-Glo™ Assay demonstrates a linear response to a serial titration of cells. **Panel A.** HepG2 cells were plated and allowed to attach overnight. The next day medium was removed, and 100µl of GSH-Glo™ Reagent was added to the cells. No centrifugation or wash steps were required. **Panel B.** A suspension cell line (Jurkat cells) was harvested, counted and resuspended in PBS. A serial twofold dilution of cells in PBS was then prepared directly in a 384-well plate. In this case, an equal volume of GSH-Glo™ Reagent 2X was added to the wells containing cells, and assays were incubated for 30 minutes. In both cases the GSH-Glo™ Assay reaction was stopped by adding an equal volume of reconstituted Luciferin Detection Reagent, and luminescence was measured after 15 minutes. Based on the GSH standard curve, the limit of detection (LOD) for HepG2 cells was approximately 45 cells and the LOD for Jurkat cells was approximately 20 cells.

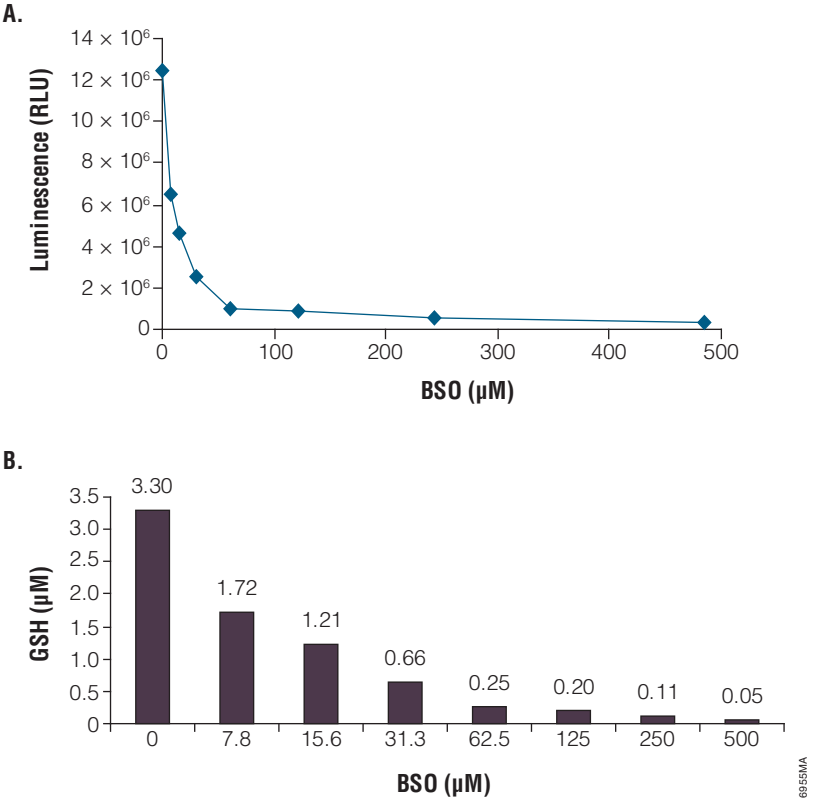

Figure 6. Treatment of HepG2 cells with L-buthionine-sulfoximine (BSO). BSO (Sigma Cat.# B-2515) inhibits GSH synthesis, thus reducing cellular GSH levels, but is nontoxic for up to 72 hours (5). HepG2 cells, at 5,000 cells/well, were plated in a 96-well plate and allowed to attach for approximately 4 hours. Various amounts of BSO were added, and the cells were incubated for 22 hours. The medium was removed from wells, and 100µl of GSH-Glo™ Reagent was added. A GSH standard curve was generated at the same time as previously described. The GSH-Glo™ Assay reaction was stopped by adding an equal volume of reconstituted Luciferin Detection Reagent, and after a 15-minute-incubation, luminescence was measured. The amount of GSH was calculated based on the standard curve. The HepG2 cells demonstrated a dramatic dose response to BSO exposure, with <20% toxicity at the highest dose as determined by parallel wells treated with BSO but assayed with CellTiter-Glo® Assay (Cat.# G7570; data not shown).

5.D. GSH-Glo™ Glutathione Assay Using a Single-Tube Luminometer

Materials to Be Supplied by the User

- Single-tube luminometer (i.e., GloMax® 20/20 Luminometer, Cat.# E5311, E5321, E5331)
- luminometer-compatible tubes

A single-tube luminometer may be used to measure the luminescent signal. The user will need to determine the optimal lysate concentration that falls within the linear range of the assay.

Harvest treated and control cells, tissue or blood lysate in PBS (containing EDTA or heparin for tissue and blood, respectively). Further dilution in PBS will be necessary and should be determined for each application.

1. Prepare GSH-Glo™ Reagent 1X as directed in Section 3.B. One hundred microliters of GSH-Glo™ Reagent 1X is needed per tube or sample for this assay. Prepare sufficient reagent just prior to use.
2. Add 10µl of diluted cells, tissue or blood lysate to the tube. The GSH standard curve can be prepared following the same method described in Section 3.B, Reagent Preparation.
3. Add 100µl of prepared GSH-Glo™ Reagent to the tube, and vortex to mix.
4. Incubate at room temperature for 30 minutes.
5. Add 100µl of prepared Luciferin Detection Reagent to each tube. Mix briefly by vortexing. Incubate for 15 minutes.
6. Measure luminescence.

5.E. GSH-Glo™ Glutathione Assay Without Medium Removal

The GSH-Glo™ Glutathione Assay is optimized for use with cells after removal of the cell culture medium used to maintain the cells. The assay also was tested with the medium present. Figure 7 shows that there is a significant loss of detectable signal in the GSH-Glo™ Glutathione Assay standard curve if medium is present in the assay. The extent of this loss depends on the medium used (Figure 7). A similar loss of detectable signal occurs in cell assays if the medium is not removed; however, the signal loss between the standard curve and cell assays in the presence of medium is roughly proportional. Therefore, data generated in the presence of medium may be used if the data are normalized to a standard curve generated in the presence of medium. Figure 8 shows that, when cellular GSH concentration is measured in 5,000 HeLa cells in the presence or absence of medium, the GSH concentrations observed are similar and the inhibitory effect of BSO is still observed.

It is unlikely that all combinations of cells and media will allow glutathione measurement without medium removal due to signal loss. Note that due to the loss of signal in the presence of medium there will be a concomitant loss in sensitivity, so performing the assay in the presence of medium may have a negative effect at lower cell numbers. Due to high levels of oxidized glutathione in most culture media, total glutathione (reduced and oxidized glutathione) in cells cannot be determined accurately without medium removal. We recommend that you test the GSH-Glo™ Glutathione Assay with the desired cells and medium in the presence and absence of medium (with 2X or 1X GSH-Glo™ Reagent, respectively) to ensure that an appropriate concentration of GSH is measured.

5.E. GSH-Glo™ Glutathione Assay Without Medium Removal (continued)

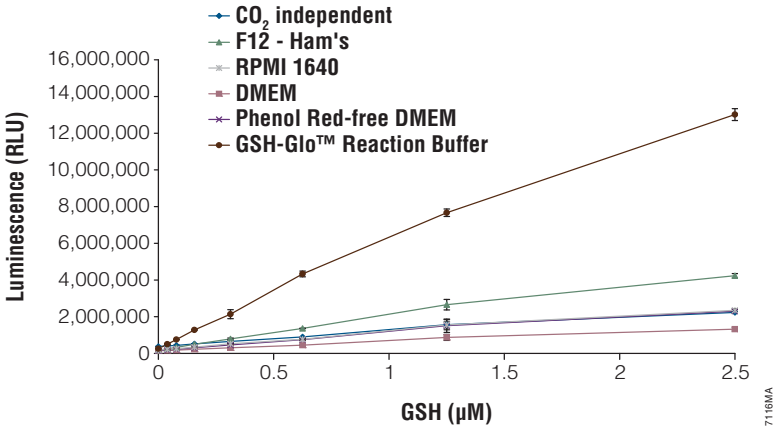

Figure 7. Comparison of GSH-Glo™ Assay results in the presence of various growth media. In a 96-well plate, 50µl of various mammalian cell growth media supplemented with 10% fetal bovine serum was added to certain wells. Additional wells were prepared with 50µl per well of GSH-Glo™ Reaction Buffer. A glutathione standard curve was generated by serial twofold dilutions of a 10X glutathione solution. Five microliters of the GSH standard solutions was added to wells containing medium or GSH-Glo™ Reaction Buffer. An equal volume (50µl per well) of GSH-Glo™ Reagent 2X was added to the wells, and assays were incubated for 30 minutes. The GSH-Glo™ Assay reaction was stopped by adding 100µl of Luciferin Detection Reagent, and luminescence was measured after 15 minutes.

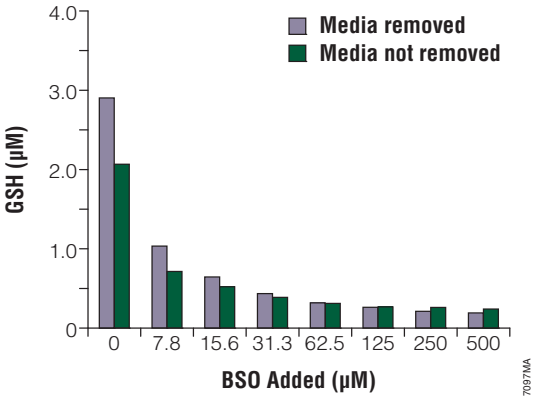

Figure 8. Comparison of GSH-Glo™ Assay results with and without medium removal. HeLa cells were plated at 5,000 cells per well in 96-well white tissue culture plates in 50µl of DMEM supplemented with 10% fetal calf serum and containing various concentrations of BSO (0–500µM). The plates were incubated overnight (22 hours at 37°C). The plates were removed from the incubator, and DMEM was removed from some wells but not from others. GSH-Glo™ Reagent 2X (50µl) was added to wells containing medium, while 100µl of GSH-Glo™ Reagent 1X was added to wells without medium. Cells were incubated for 30 minutes, and then Luciferin Detection Reagent was added. Plates were incubated and luminescence measured as described in Section 3.C.

5.F. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com.** E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
GSH standard curve not linear	Brightness of standards exceeded the linear range. Linear regression showed an $r^2 < 0.97$. Adjust gain settings on luminometer to accommodate brighter signals. Dilute test samples to bring signals within the linear range of instrument.
	Linear regression showed an $r^2 < 0.97$, resulting in higher than targeted GSH concentrations. Check that GSH dilutions were correct. The final GSH standard concentrations, after adding the Luciferin Detection Reagent, should be 5.0, 2.5, 1.25, 0.625, 0.31, 0.015 and 0µM.
High background luminescence	Luciferin contamination in one or more of the reaction components: • Avoid workspaces and pipettes that are used with luciferin-containing solutions, including luminescence-based cell viability, apoptosis or gene reporter assays • Decontaminate work surfaces by wiping with a detergent solution or ethanol and rinsing with clean water. Rinse pipettes and other labware with distilled water at least three times. For automated dispensing systems, replace any components that were used to dispense luciferin-containing solutions.
	Substrate was stored improperly. Luciferin-NT should be stored at –20°C, protected from light.
	GSH-Glo™ Reagent was prepared and stored before use. Prepare GSH-Glo™ Reagent (GSH-Glo™ Reaction Buffer + GST Enzyme + Luciferin-NT substrate) immediately before use. Do not store reagent for future (or further) use.
Low luminescent signal	Use only white, opaque luminometer plates. Do not use black plates or clear plates.
	Avoid multiple freeze-thaw cycles of the reconstituted Luciferin Detection Reagent.

5.F. Troubleshooting (continued)

Symptoms	Causes and Comments
Unexpected inhibition of luciferase or GST Enzyme	The GST Enzyme catalyzes the first step of the GSH-Glo™ Reaction, and luciferase is used to generate luminescence in the second step of the GSH-Glo™ Assay. The potential for inhibition of luciferase or GST Enzyme is minimized by maintaining high enzyme concentrations and using reaction chemistries that reduce the effects of potential inhibitors.
	To test for luciferase inhibition, assemble two reactions: one with equal volumes of reconstituted Luciferin Detection Reagent and 400nM Beetle Luciferin, Potassium Salt (Cat.# E1601) and a second reaction with equal volumes of reconstituted Luciferin Detection Reagent and 400nM beetle luciferin plus the test compound. Incubate reactions for 10 minutes at room temperature, then measure luminescence. A decrease in luminescence in the presence of test compound is an indication of luciferase inhibition.
	If luciferase inhibition was ruled out, perform the GSH-Glo™ Assays without test compound to check for GST Enzyme inhibition. Add Luciferin Detection Reagent to a control reaction and Luciferin Detection Reagent plus the test compound to a test reaction. Diminished signal in the test reaction indicates GST enzyme inhibition.

5.G. Related Products

Viability Assays

Product	Size	Cat.#
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
CellTiter-Glo® 2.0 Assay	10ml	G9241
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
CellTiter-Blue® Cell Viability Assay	20ml	G8080

Other sizes are available.

Cytotoxicity Assays

Product	Size	Cat.#
CellTox™ Green Cytotoxicity Assay	10ml	G8741
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
CytoTox-Fluor™ Cytotoxicity Assay	10ml	G9260

Other sizes are available.

Multiplex Viability and Cytotoxicity Assays

Product	Size	Cat.#
MultiTox-Glo Multiplex Cytotoxicity Assay	10ml	G9270
MultiTox-Fluor Multiplex Cytotoxicity Assay	10ml	G9200

Other sizes are available.

Mechanism-Based Viability and Cytotoxicity Assays

Product	Size	Cat.#
ApoTox-Glo™ Triplex Assay	10ml	G6320
ApoLive-Glo™ Multiplex Assay	10ml	G6410

Other sizes are available.

Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	10ml	G8091
Caspase-Glo® 8 Assay	10ml	G8201
Caspase-Glo® 9 Assay	10ml	G8211
Apo-ONE® Homogeneous Caspase-3/7 Assay	10ml	G7790

Other sizes are available.

Promega

5.G. Related Products (continued)

Metabolism Assays

Product	Size	Cat.#
NAD(P)H-Glo™ Detection System	10ml	G9061
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081

Other sizes are available.

Mitochondrial Toxicity

Product	Size	Cat.#
Mitochondrial ToxGlo™ Assay	10ml	G8000
	100ml	G8001

Oxidative Stress Assays

Product	Size	Cat.#
ROS-Glo™ H_2O_2 Assay	10ml	G8820
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611

Other sizes are available.

Cytochrome P450 Cell-Based Assays

Product	Size	Cat.#
P450-Glo™ CYP1A2 Induction/Inhibition Assay	10ml	V8421
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	10ml	V9001
P450-Glo™ CYP2C9 Assay	10ml	V8791

Other sizes are available.

Detection Instrumentation

Product	Size	Cat.#
GloMax® Discover System	each	GM3000

5.H. Summary of Changes

The following changes were made to the 3/15 revision of this document:

1. The legal disclaimers were updated.