

# GSH-Glo™ Glutathione Assay: Novel Luminescent Assay to Assess Changes in Glutathione Levels in Cells.

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## 1. Abstract

Glutathione, (GSH) a non-protein thiol, is an antioxidant abundantly found in eukaryotic cells. Reactive chemical species and drug-drug interactions often cause a drop in GSH levels either by oxidation or reaction with the thiol group. Therefore measurement of GSH is important in the assessment of toxicological responses.

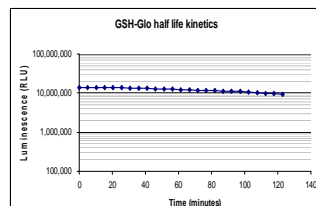
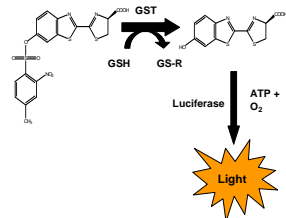
GSH-Glo™ is a luminescent assay for the detection and quantification of GSH in cultured cells and other biological samples. The GSH-Glo™ assay format is amenable to high throughput screening making this new method a valuable tool for drug discovery and pharmaceutical applications.

## 2. Assay Concept

The 2-step assay, GSH-Glo™, is based upon the conversion of a luciferin derivative into luciferin in the presence of glutathione, catalyzed by glutathione-S-transferase [GST] enzyme. The light signal generated in the coupled reaction with firefly luciferase is proportional to the amount of glutathione present in the sample.

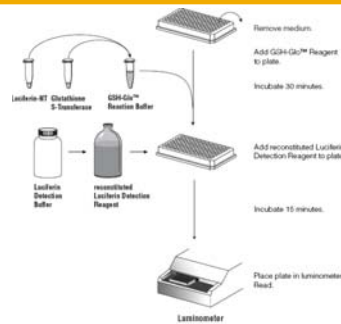
This assay has been optimized for mammalian cells in 96 well plates. It can also be used in other formats and with a variety of different biological samples.

## 3. Assay Chemistry and Glo Kinetics

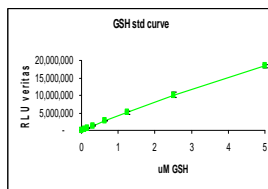


Graph shows the luminescent signal from 5uM GSH in a cell-free 96well assay format. The luminescent signal half life is ~ 4 hours.

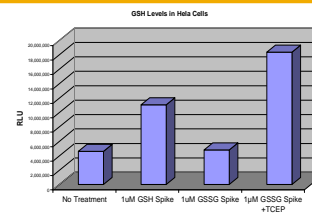
## 4. Steps in the GSH-Glo™ Glutathione Assay



## 5. Limit of Detection and Specificity



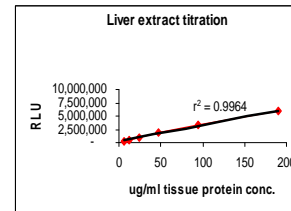
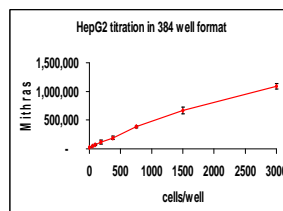
The inclusion of a GSH Standard solution facilitates the conversion of RLU to GSH concentration. The limit of detection is approximately 1-5nM GSH.



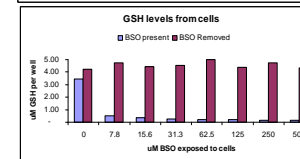
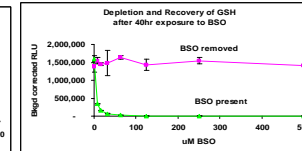
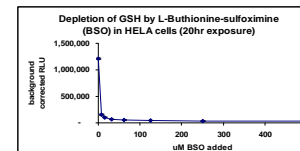
The GSH-Glo assay measures **reduced** glutathione; it is unaffected by the presence of oxidized glutathione. Total glutathione levels in can be determined after by the addition of a reducing reagent that converts GSSG to GSH (i.e., TCEP)

## 6. Sensitivity in mammalian cell and tissue lysates

The assay is very sensitive and can easily detect less than 1000 cells in a 96 well format or 300 cells in a 384 well format. Additionally, GSH can be detected from <10mg of tissue extract and < 100ul of blood lysate.



## 7. Treatment of cells with BSO to deplete GSH



Cells show a dose dependent drop in RLU after overnight exposure to BSO. Subsequent removal of BSO and replenishment with fresh media allows the GSH levels to return to baseline levels.

## 8. Summary

- Optimized for determining reduced Glutathione levels in mammalian cells
- GSH-Glo can be used to measure total glutathione
- Highly Sensitive- Requires many fewer cells per assay than competing systems
- Fast and Easy to use- less steps
- No deproteination step required
- Targeted user group: ADME Tox staff in Pharmaceutical companies, and university researchers in the Pharmacology and Toxicology Departments.

## 9. GSH-Glo™ Glutathione Assay

Cat#.	Kit Sizes
V6911	10ml
V6912	50ml

### Kit Components

- V687A/B Luciferin-NT: 100ul and 500ul
- V688A/B GSH-Glo Reaction Buffer: 10ml and 50ml
- V689A/B Glutathione S-Transferase: 100ul and 500ul
- V690A Glutathione, 5mM: 100ul
- V144A/B Luciferin Detection Buffer: 10ml and 50ml
- V859A/B Luciferin Detection Reagent: 1 bottle each size