HOMOGENEOUS LUMINESCENCE-BASED ASSAY FOR QUANTIFYING THE GLUTATHIONE CONTENT IN MAMMALIAN CELLS

CHRISTIANE SCHERER, SILVIA CRISTOFANON, MARIO DICATO, MARC DIEDERICH*,LABORATOIRE DE BIOLOGIE MOLÉCULAIRE ET CELLULAIRE DU CANCER, HÔPITAL KIRCHBERG, 9, RUE EDWARD STEICHEN, L-2540 LUXEMBURG, LUXEMBURG, *CORRESPONDING AUTHOR. TEL: +352 2468 4040; FAX: +352 2468 4060; E-MAIL ADDRESS; MARC.DIEDERICH@LBMCC.LU

Promega has developed a new luminescence-based assay to rapidly quantify the total intra- and extracellular glutathione (GSH) content of mammalian cells and tissues. This method is able to rapidly assess the basic level of this antioxidant, nonprotein thiol in different eukaryotic cell lines. In addition, the antioxidant or prooxidant activity of diverse xenobiotics affecting cellular glutathione levels can be investigated with little effort. Hence we describe experiments with the human leukemic cell line U937, where the intracellular glutathione biosynthesis/level is induced or reduced after treatments with N-acetylcysteine (NAC) or buthionine-(S,R)-sulfoxime (BSO), respectively.

Introduction

During the last decades it has become apparent that physiological levels of reactive oxygen species (ROS) play an important role in various diseases, such as chronic inflammation and cancer (1). One factor that can modulate ROS level is the nonprotein thiol glutathione (GSH) that is produced by mammalian cells (2). This antioxidant can interact with diverse ROS species and is consequently oxidized itself, thereby generally reducing cellular redox stress. Additionally, biosynthesis of GSH can be up- or downregulated by the cells when triggered by a huge number of physiological and xenobiotic compounds (Figure 1). An imbalance of ROS can lead to protection or physiological stress of the cells, which can in the latter case induce manifold cellular defense mechanisms frequently leading to cell death, partially by apoptosis (3). Furthermore if GSH level is decreased, the capacity of cells for detoxification by phase II metabolism is reduced (Figure 1). Hence it is important to evaluate the mechanisms of action of different natural agents during preclinical drug development by investigating alterations in GSH levels.

Reagents, Materials and Instrumentation

In the experiments described here, we used the GSH-Glo™ Glutathione Assay Kit (Cat.# V6911, V6912), buthionine-(S,R)-sulfoxime (BSO; Sigma), N-acetylcysteine (NAC;

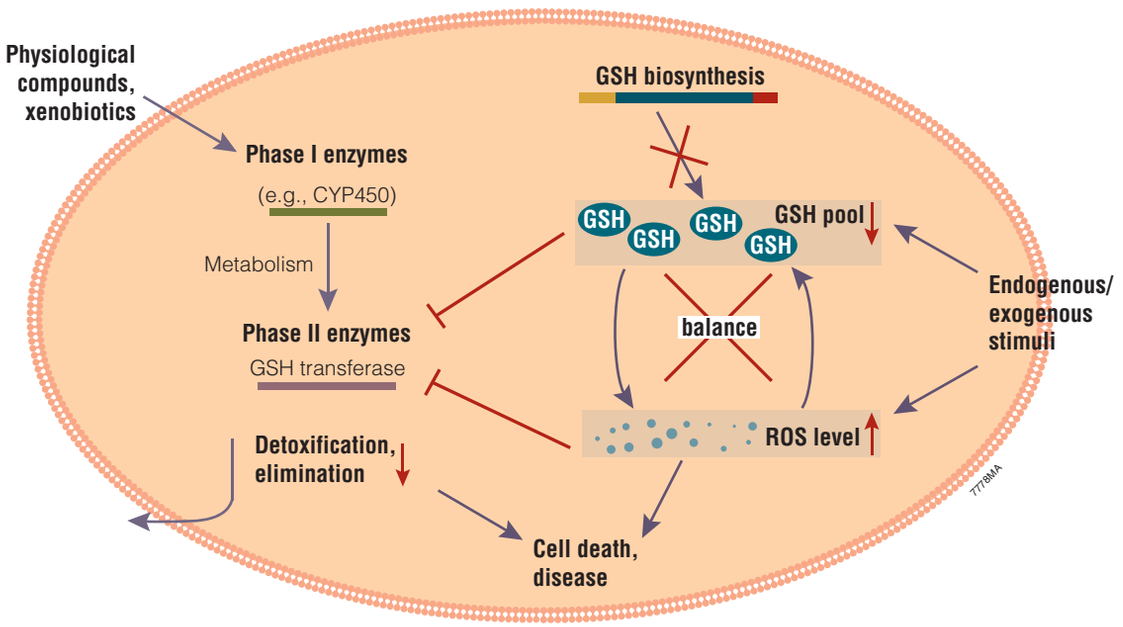


Figure 1. Schematic of the physiological roles of glutathione (GSH).

GSH ASSAY

Sigma), Orion Microplate Luminometer (Berthold), and 96-well luminometer plates (Greiner Bio-one).

Cell Culture

U937 cells (human histiocytic lymphoma, DSMZ) were cultured in RPMI 1640 medium (Bio-Whittaker) containing 10% [v/v] fetal calf serum (FCS; Cambrex) and 1% [v/v] antibiotic-antimycotic (Bio-Whittaker) at 37 °C, 5% CO_2.

Generating Glutathione Standard Curve

To calibrate the test system, a glutathione standard curve was generated according to the *GSH-Glo™ Glutathione Assay Technical Bulletin* #TB369. Serial dilutions of a 5 mM stock solution of glutathione were performed in PBS (range was 0–5 µM), then 10 µl of these solutions was put in triplicate wells of 96-well luminometer plates; subsequently 100 µl of GSH-Glo™ Reagent was added to each well, and the plate was incubated for 30 minutes at 22 °C in the dark. Afterwards, 100 µl of reconstituted Luciferin Detection Reagent was added to each well, and luminescence was measured after 15 minutes of incubation. Luminescence measurements were performed using a 1-second integration period.

For our setup, a plot of GSH concentration versus luminescence, measured as relative light units (RLU; Figure 2), was linear between 0.5 and 5 µM, as expected (correlation coefficient >0.99).

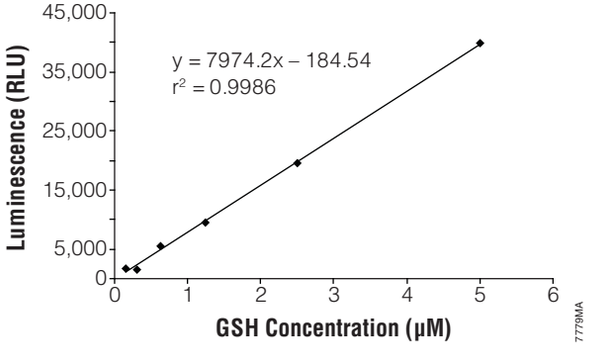


Figure 2. Function of GSH concentration and luminescence Y-axis: luminescence, X-axis: glutathione concentration. Relative S.D. <10%.

Determining Intracellular Glutathione Content of U937 Cells

U937 cells were used as a model system for mammalian cells in suspension. Cells were harvested by centrifugation, washed, resolubilized and diluted in PBS; then 1,000–2,000 cells in 50 µl were added to triplicate wells. The 2X GSH-Glo™ Reagent (50 µl) was added to each well, the plate was mixed on an orbital shaker for 1 minute and incubated for 30 minutes at 22 °C in the dark. Following incubation, 100 µl of prepared Luciferin Detection Reagent was added to each well, samples were mixed briefly on an orbital shaker, and luminescence was measured after 15 minutes of incubation. Luminescence measurements were performed using a 1-second integration period.

For our setup, the linear range of the function of the intracellular GSH concentration and the corresponding luminescence units (RLUs) (Figure 3) was linear between 2,000–8,000 cells (correlation coefficient >0.99).

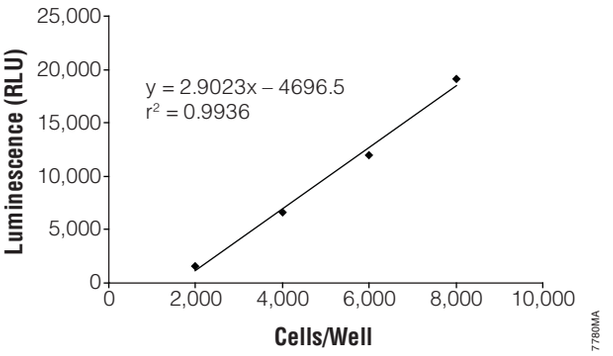


Figure 3. Function of intracellular GSH concentration of U937 cells and luminescence. Y-axis: luminescence, X-axis: number of U937 cells per well quantified by their intracellular glutathione content. Relative S.D. <12%.

Modulation of Cellular Glutathione Level

To reduce biosynthesis of GSH, U937 cells were treated with 1 mM of buthionine-(S,R)-sulfoxime (BSO) for 24 hours; to induce biosynthesis of the thiol, cells were exposed to 10 mM of N-acetylcysteine (NAC) for 48 hours. To quantify the glutathione content, 4,000 cells were added to each well, and analyses were performed as described in the previous section.

We found that the intracellular gluthatione level of U937 cells was strongly reduced after BSO treatment, whereas it clearly increased after incubation with NAC (Figure 4).

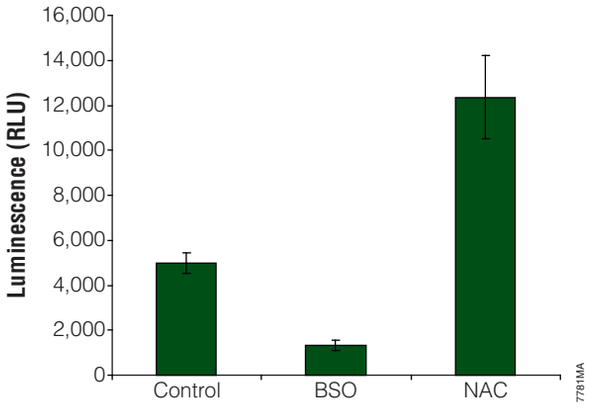


Figure 4. Modulation of intracellular GSH concentration of U937 cells by BSO or NAC. Y-axis: luminescence, X-axis: samples (control: nontreated control, BSO: buthionine sulfoxime-treated cells, NAC: N-acetylcysteine-treated cells).

Summary

We confirmed that the GSH-Glo™ Assay produced linear results in the GSH concentration range (0.5–5 µM) indicated by Promega. We also determined the optimal range of U937 cell numbers for a linear correlation between intracellular GSH concentration and luminescence. Finally, we showed the applicability of the assay system by comparing the luminescence of nontreated cells with the luminescence of cells with an increased (NAC-treated) or reduced (BSO-treated) intracellular GSH content.

The GSH-Glo™ Assay system is an easy-to-use assay to quantify glutathione in our model. The assay could be used as a screening system to assess effects of different compounds on ROS linked with GSH.

References

1. Davenport, D.M. and Wargovich M.J. (2005). *Food Chem. Toxicol.*, **43**, 1753–62.
2. Dröge, W. (2002) *Physiol. Rev.* **82**, 47–95.
3. Kim, H.J. *et al.* (2006) *Oncogene* **25**, 2785–94.

Protocols

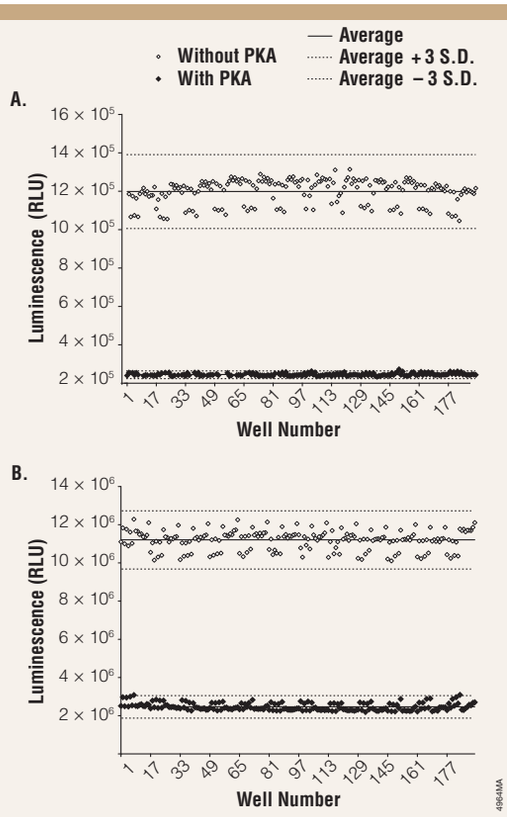
GSH-Glo™ Glutathione Assay Technical Bulletin #TB369 (www.promega.com/tbs/tb369/tb369.html)

Ordering Information

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

ERRATUM

The article “Screen for Kinase Modulators in a High-Throughput Format with Promega Kinase Reagents” (pages 21–24 in *Cell Notes* Issue 20) contained an error in the legend for Figure 4. The Z′-factor values in the experiment described were determined for the Kinase-Glo® Max Assay, not the Kinase-Glo® Plus Assay. The figure with corrected legend is presented here.



Determining Z′-factor for Kinase-Glo® Max Assay run in a 384-well plate. **Panel A.** The assay was performed as described in Technical Bulletin #TB372 with 0.2 units/well PKA and 10 µM ATP for five minutes at room temperature (solid symbols) or without PKA (open symbols). **Panel B.** The assay was performed using 0.2 units/well PKA and 100 µM ATP for 30 minutes at room temperature (solid symbols) or without PKA (open symbols). Assays were performed in 384-well plates in a final volume of 20 µl. Solid lines indicate the mean, and the dotted lines indicate ± 3 S.D. Z′-factor values were 0.8 for both 10 µM and 100 µM ATP.