Glutathione Cell-Based Detection Kit (Blue Fluorescence)

Item No. 600360

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600361	Cell-Based Assay Monochlorobimane	1 vial/100 µl	-20°C
600362	Cell-Based Assay Glutathione Standard	1 vial	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	RT
10010215	Cell-Based Assay Lysis Buffer	1 vial/10 ml	RT
10011297	96-Well Solid Plate (black) with lid	1 plate	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the capacity to measure fluorescence at excitation and emission wavelengths of 380 nm and 480 nm, respectively
2. A 96-well plate for culturing cells
3. Adjustable pipettes and a repeating pipettor
4. A source of pure water; glass distilled water or HPLC-grade water is acceptable
5. A plate centrifuge

INTRODUCTION

Background

Glutathione (GSH) is the most prevalent low molecular-weight tripeptide thiol in animal cells.[1] Living cells internally generate oxygen radicals and hydrogen peroxide (H_2O_2), both of which cause massive oxidative stress. GSH is the major anti-oxidant which scavenges oxygen radicals and H_2O_2.[2] In addition, GSH binds to certain environmental toxins such as heavy metals in low quality foods, polluted air, or in drinking water to form water-soluble conjugates that are ultimately excreted in the urine or bile as waste. Thus, GSH protects cells from multiple assaults from oxygen radicals, H_2O_2, or environmental toxins.[3] Deprivation of GSH results in oxidative damage associated with mitochondrial degeneration.[4] The age-related decrease in GSH plays an important role in the aging process and in oxidative stress-related disease.[5] During early apoptosis, cells may exclude reduced GSH, causing a decrease in intracellular GSH levels.[6] The intracellular level of GSH is thus used as an indicator of cell health.

About This Assay

Cayman's Glutathione Cell-Based Assay Kit (Blue Fluorescence) employs a cell-permeable dye, monochlorobimane (MCB), which reacts with GSH to generate a highly fluorescent product that can be measured using excitation and emission wavelengths of 380 and 480 nm, respectively.[7] The kit can be easily adapted to high-throughput screening for therapeutic compounds regulating intracellular GSH levels. GSH is included in the kit for use as a positive control or as a standard.

PRE-ASSAY PREPARATION

Reagent Preparation

Cell-Based Assay Buffer Preparation

Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

Substrate Solution Preparation

Add 50 µl of the Cell-Based Assay Monochlorobimane (Item No. 600361) to 1 ml of Cell-Based Assay Buffer prepared above.

Preparation of Standard

Dissolve the contents of the Cell-Based Assay Glutathione Standard (Item No. 600362) in 1 ml of Cell-Based Assay Buffer. The concentration of this diluted GSH Standard will be 10 mM. This GSH Standard is stable for four weeks at 4°C.

To use the GSH Standard as a positive control, dilute 1:100 in Cell-based Assay Buffer. Load 90 µl of this 0.1 mM solution into control wells in the assay plate.

To run a GSH standard curve, obtain eight clean test tubes and label them #1 to #8. Add 975 µl of Assay Buffer into tube #1 and 250 µl into tubes #2-#8. Transfer 25 µl of GSH Standard from the 10 mM GSH Standard Solution into tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, is 250 µM. Serially dilute the standard by removing 250 µl from tube #1 and placing it into tube #2; mix thoroughly. Next remove 250 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this procedure for tubes #4 to #7. Do not add any standard to tube #8. This tube will be your blank.

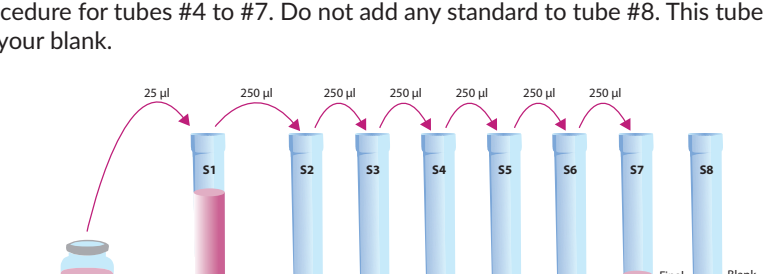


Figure 1. Preparation of the GSH Standard Curve

ASSAY PROTOCOL

Whole Cell Staining

1. Culture cells in 6-, 12-, 24-, or 96-well plates at a density of 5 x 10^5 cells/ml in a CO_2 incubator overnight at 37°C. Treat the cells with or without experimental compounds (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Add 100 µl of Substrate Solution (prepared on page 8) per ml of culture medium to each well of the plate. Mix gently. Further dilution of the Substrate Solution may be used if the staining is too intense.
3. Incubate samples in a CO_2 incubator at 37°C for 15-30 minutes. Sufficient staining is usually obtained after 15 minutes of incubation.
4. Centrifuge the plate in a plate centrifuge at 1,000 rpm for five minutes.
5. Discard the supernatant by careful aspiration.
6. Add 2 ml, 1 ml, 500 µl, or 200 µl of Assay Buffer to each well of 6-, 12-, 24-, or 96-well plate, respectively.
7. The cells are now ready for analysis. We recommend using a microscope or plate reader for analysis of adherent cells, and excitation and emission wavelengths of 380 nm and 480 nm, respectively. If a flow cytometer with a UV or violet laser and a filter around 450 nm is available, this is the best way to analyze suspension cells.

Cell Lysate Measurement

1. Seed cells in a 96-well plate at a density of 1 x 10^5-5 x 10^5 cells/well in 200 µl of culture medium. Incubate overnight in a CO_2 incubator at 37°C. The next day, treat the cells with compounds to be tested or vehicle and continue to culture the cells at 37°C for a period of time according to your protocol. We recommend that you perform triplicate wells for each treatment.
2. Centrifuge the plate in a plate centrifuge at 1,000 rpm for five minutes.
3. Aspirate the culture medium.
4. Add 100 µl of Cell-Based Assay Lysis Buffer (Item No. 10010215) to each well.
5. Incubate with gentle shaking on an orbital shaker for 15 minutes at room temperature.
6. Centrifuge the plate at 1,000 rpm for 10 minutes. Transfer 90 µl of the cell lysate supernatant from each well to a corresponding well in the black 96-well plate included in the kit.
7. Add 90 µl of GSH standard or positive control into the corresponding wells of the black plate.
8. Add 10 µl of the Substrate Solution to each well and incubate the plate at room temperature for 1.5-2 hours.
9. Read the fluorescence intensity of each well (excitation = 380 nm; emission = 480 nm).

ANALYSIS

Calculations

Plot the Standard Curve

Make a plot of Fluorescence as a function of GSH concentration and determine the equation of the line. See Figure 4, on page 14, for a typical standard curve.

Determination of GSH Concentration

GSH concentration (µM) = [Fluorescence - (y-intercept)]/Slope

NOTE: It is not necessary to convert fluorescence to GSH concentration, especially when you are only looking at the difference between controls and treatments. Since factors such as cell density, cell health, and timing of treatments differ from experiment to experiment, cautions should be taken when comparing results from different experiments.

Performance Characteristics

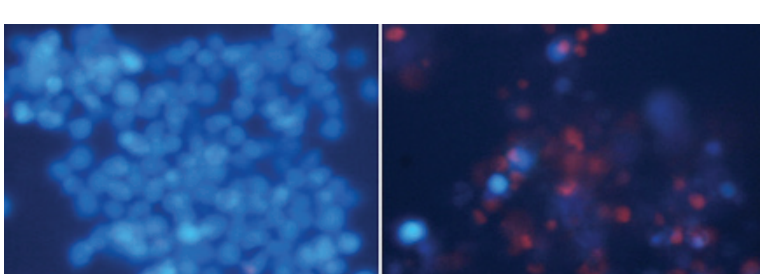


Figure 3. H_2O_2 reduces GSH levels in Jurkat cells. Jurkat cells were seeded in a 96-well plate in 100 µl of culture medium at a density of 1 x 10^5 cells/well and were incubated at 37°C in a CO_2 cell culture incubator overnight. The next day, cells were treated with 200 µM H_2O_2 for 24 hours. Cells were then processed for staining according to the whole cell staining protocol on page 10. Dead cells were visualized by staining with Propidium Iodide (Item No. 10011234). *Panel A:* control, untreated cells have homogenous GSH staining throughout the cells and few cells are propidium iodide positive. *Panel B:* treatment of cells with 200 µM H_2O_2 for 24 hours causes significant cell death and loss of GSH staining.

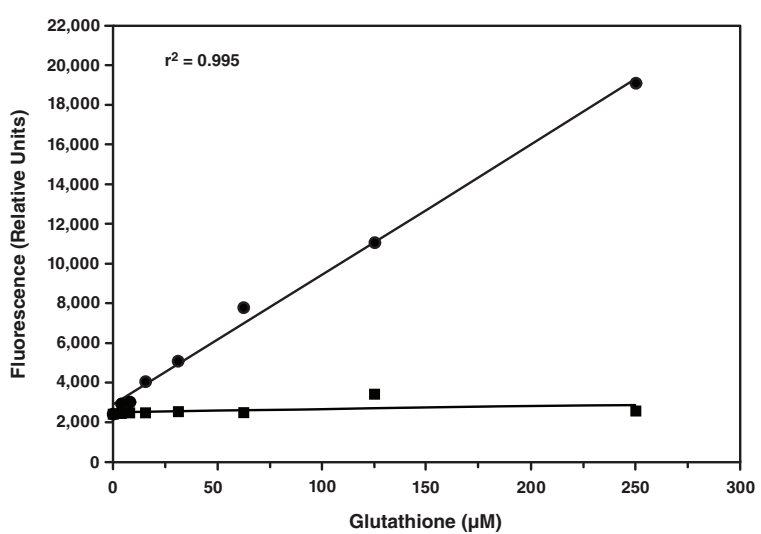


Figure 4. Cell-Based Glutathione Standard Curve
The standard curve presented here is an example of the data typically produced with the assay. However, your results will not be identical to these. You must run a new standard curve for each experiment.

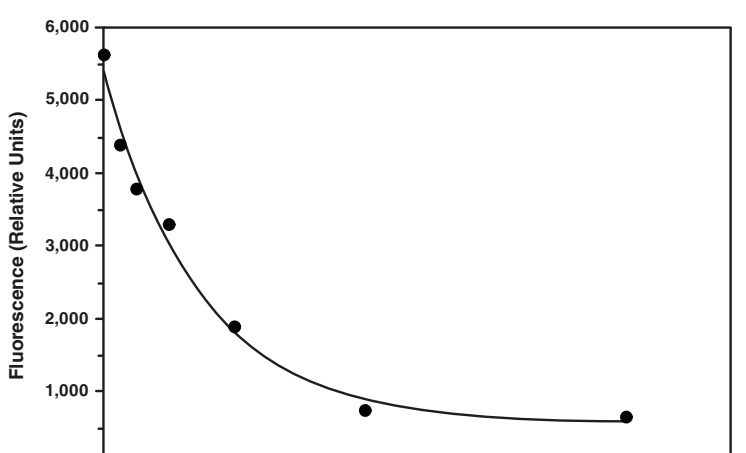


Figure 5. Staurosporine treatment reduces GSH levels in HeLa cells. HeLa cells were seeded in a 96-well plate in 200 µl of culture medium at a density of 1 x 10^5 cells/well and were incubated at 37°C in a CO_2 cell culture incubator overnight. The next day, cells were treated overnight in a 37°C incubator with different doses of staurosporine as indicated. Cells were then processed for measurement of GSH according to the protocol described in the **Cell Lysate Measurement** section.

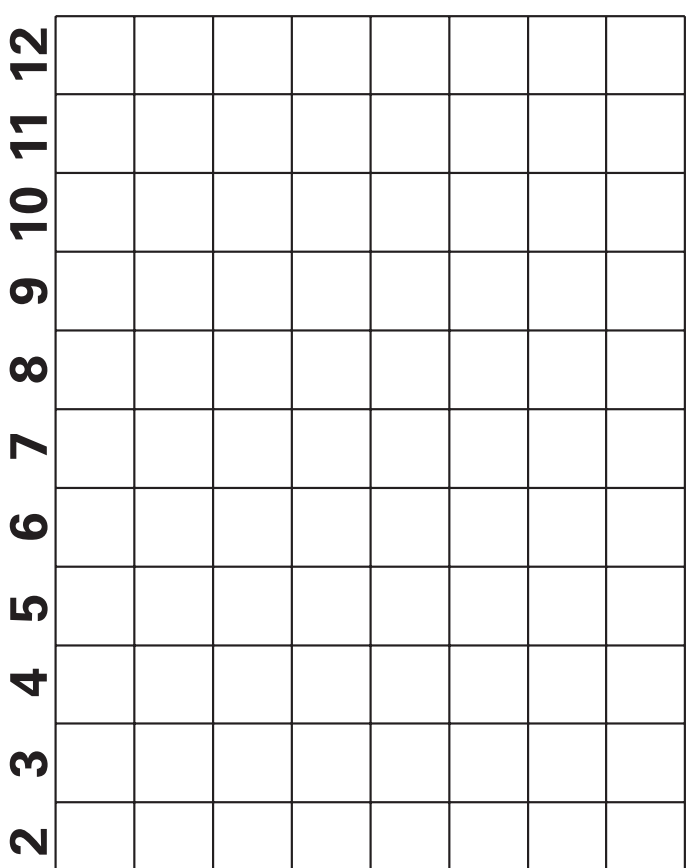
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Erratic response curve of compound treatments	Cells lost from wells during processing, or unequal number of cells in each well	Do triplicate wells for each treatment; use only healthy cells at the beginning of the experiment; make sure each well contains the same number of cells

References

1. Martin, H.L. and Teismann, P. Glutathione--A review on its role and significance in Parkinson's disease. *FASEB J.* **23**, 3263-3272 (2009).
2. Hayes, J.D. and McLellan, L.I. Glutathione and glutathione-dependent enzymes represent a coordinately regulated defence against oxidative stress. *Free Radic. Res.* **31**, 273-300 (1999).
3. Dickinson, D.A. and Forman, H.J. Cellular glutathione and thiols metabolism. *Biochem. Pharmacol.* **64**, 1019-1026 (2002).
4. Jain, A., Mårtensson, J., Stole, E., *et al.* Glutathione deficiency leads to mitochondrial damage in brain. *Proc. Natl. Acad. Sci. USA* **88**, 1913-1917 (1991).
5. Erden-Inal, M., Sunal, E., and Kanbak, G. Age-related changes in the glutathione redox system. *Cell Biochem. Funct.* **20**, 61-66 (2002).
6. Macho, A., Hirsch, T., Marzo, I., *et al.* Glutathione depletion is an early and calcium elevation is a late event of thymocyte apoptosis. *J. Immunol.* **158**, 4612-4619 (1997).
7. Rice, G.C., Bump, E.A., Shrieve, D.C., *et al.* Quantitative analysis of cellular glutathione by flow cytometry utilizing monochlorobimane: Some applications to radiation and drug resistance *in vitro* and *in vivo*. *Cancer Res.* **46**, 6105-6110 (1986).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.