For research use only

Glutathione Assay Kit (GSH, GSSG and Total)

(Catalog #K264-100; 100 assays; Store kit at -20°C; Lot #: 40664)

I. Introduction:

Glutathione is the major intracellular low-molecular-weight thiol that plays a critical role in cellular defense against oxidative stress in tissues and cells. Commercially available glutathione detection kits, such as the DTNB-enzyme cycling glutathione assay kit or the Monochlorobimane based assay kit hardly distinguish between reduced glutathione (GSH; FW: 307) and oxidized glutathione (GSSG; FW: 612). BioVision's Glutathione Detection Kit provides a unique, convenient tool for detecting GSH, GSSG, and total glutathione individually. In the assay, OPA, reacts with GSH (not GSSG), generating fluorescence, so GSH can be specifically quantified. Adding a reducing agent converts GSSG to GSH, so (GSH + GSSG) can be determined. To measure GSSG specifically, a GSH Quencher is added to remove GSH, preventing reaction with OPA (while GSSG is unaffected). Reducing agent is then added to destroy excess quencher and to convert GSSG to GSH. Thus, GSSG can be specifically quantified. The kit provides a unique procedure and buffer formula to eliminate protein thiol interference and to stabilize GSH and GSSG in solution. The assay is easy to perform and detects 2-400 ng/µl of GSH, GSSG or total glutathione.

II. Kit Contents:

Kit Component	100 Assays	Cap Code	Part Number
Glutathione Assay Buffer	30 ml	WM	K264-100-1
PCA (Perchloric Acid, 6N)	2 ml	Red	K264-100-2
KOH (3N)	2 ml	Blue	K264-100-3
OPA Probe (o-phthalaldehyde)	0.2 ml	Brown	K264-100-4
Reducing Agent Mix	1 vial	Green	K264-100-5
GSH Quencher	20 µl	Purple	K264-100-6
GSH Standard (FW: 307)	1 mg	Yellow	K264-100-7

III. Reagent Reconstitution and Storage:

OPA Probe, Reducing Agent Mix, GSH Quencher: Add 0.85 ml H2O to the OPA probe, mix. Dissolve Reducing Agent, GSH Quencher in 1.05 ml of dH2O separately. Store at –20°C. Use within two months.

GSH Standard: Accurately dissolve in 45 µl dH2O and add 5 µl PCA to stabilize the standard GSH stock solution (20 µg/µl). Store at –20°C. Use within two months.

IV. Sample Collection and Storage*:

Tissue and Cell Samples:

GSH is labile and cell preparations will oxidize it rapidly. Keep all samples and reagents ice cold and work as rapidly as possible. Prepare centrifuge tubes with 20µl PCA on ice to receive samples. Homogenize 2-4 x10^6 cells or 40 mg tissue on ice with 100 µl of ice cold Glutathione Assay Buffer. Take 60 µl of each homogenate to a prechilled tube containing PCA and vortex several seconds to achieve a uniform emulsion. Keep on ice for 5 min. Spin 2 min at 13,000 G at 4°C, collect supernatant (containing glutathione) and discard the protein pellet. The sample can then be stored at –80°C, stable for a month.

Serum or Other Liquid Samples:

Freeze samples immediately upon acquisition and keep frozen until ready for processing. Take 60 µl thawed sample to centrifuge tube containing 20 µl ice cold PCA, vortex and keep on ice for 5 min. Spin for 2 min at 13,000 G at 4°C. Collect the supernatant. The sample can then be stored at –80°C, stable for a month.

V. Assay Protocol:

1. **Standard Curve:** Add 10 µl of the 20 µg/µl standard GSH stock to 990 µl of Assay Buffer to generate 0.2 µg/µl working standard solution. Add 0, 2, 4, 6, 8, 10 µl to a 96-well plate to generate 0, 0.4, 0.8, 1.2, 1.6, 2.0 µg/well GSH. Bring the volume to 90 µl with Assay Buffer. **Note**: If the concentration of your assay samples is lower than the above standard range, the standard can be further diluted 10-fold to generate 0, 40, 80, 120, 160, 200 ng/well GSH by following the same procedure.
2. **Preparation of Samples for Assays:** Add 20 µl of ice cold 3N KOH to 40 µl of PCA preserved samples (as prepared in Section IV) to precipitate PCA and neutralize the samples (pH should be 5-10). Keep on ice for 5 min then spin 2 min at 13,000 G at 4°C. Transfer 10 µl of the neutralized samples to a 96-well plate. You may choose to add several dilutions (e.g., 1-10 µl) of your samples to ensure the readings are within the standard curve range.
   - **A. To Detect GSH:** Bring the sample volume to 90 µl with Assay Buffer.
   - **B. To Detect Total Glutathione:** Bring the sample well to 80 µl with Assay Buffer. Do a background control without sample. Add 10 µl of Reducing Agent Mix to the wells, mix well, incubate at room temperature for 10 min to convert GSSG to GSH.
   - **C. To Detect GSSG:** Bring the sample well volume to 70 µl with Assay Buffer. Do a background control without sample. Add 10 µl of GSH Quencher, mix well, and incubate at room temperature for 10 min to quench GSH. Then add 10 µl of Reducing Agent Mix to destroy the excess GSH Quencher and convert GSSG to GSH.
3. **Assay:** Add 10 µl of OPA Probe into the standard and sample wells, mix well, incubate at room temperature for 40 min. Read samples and standards on a fluorescence plate reader equipped with Ex/Em = 340/420 nm. We suggest adjusting the plate reader settings so that the background reading without glutathione is at about 50-150 RFU.
4. **Calculations:** Subtract background reading from sample readings. Plot RFU vs GSH standard. Apply the sample readings to the standard curve to get glutathione amount in each sample.

Glutathione Concentration = Ga/Sv

Where Ga: Glutathione amount from standard curve.

Sv: Sample volume added to the sample wells (Note: The original samples have been diluted 2-fold by performing the procedures above).

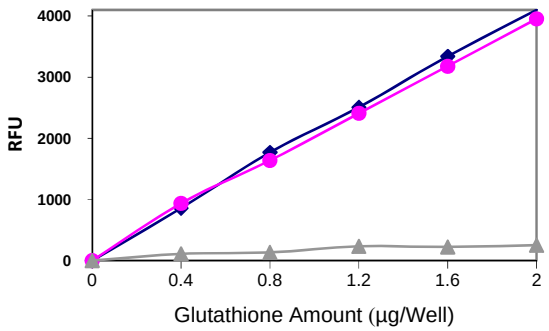

Fig 1. G SSG following kit instructions. Diamond (blue) represents GSH standard curve. Circle (pink) represents standard curve produced by adding GSSG + GSH Quencher + Reducing Agent Mix. Triangle (gray) represents GSSG standard without adding Reducing Agent mix.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85