Datasheet for ABIN2344954

OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit

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Overview

Quantity:	100 tests
Reactivity:	Others
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The OxiSelect™ Total Glutathione Assay Kit is a quantitative assay for measuring the total glutathione content within a sample (GSH/GSSG).
Brand:	OxiSelect™
Sample Type:	Cell Culture Lysate, Cell Lysate, Plasma, Saliva, Serum, Urine
Analytical Method:	Quantitative
Sensitivity:	4 nM
Characteristics:	OxiSelect™ Total Glutathione Assay Kit is a quantitative assay for measuring the total concentration of glutathione, which encompasses both reduced and oxidized glutathione (GSH and GSSG) from plasma, blood, saliva, urine, tissue extracts, and plant or mammalian cell lysates. The kit employs a simple enzymatic recycling reaction for total glutathione quantification. The kit has a detection sensitivity limit of 8 nM. Each kit provides sufficient reagents to perform up to 100 assays, including standard curve and unknown samples.
Components:	1. Glutathione Reductase (50X) : One 50 µL amber tube. 2. Chromogen (15X) : One 1 mL amber tube. 3. Assay Buffer (5X) : One 50 mL bottle. 4. Metaphosphoric Acid (MPA) : One 2 g bottle of crystals. 5. Glutathione Disulfide (GSSG) : One 50 µL amber tube of a 1 mM solution.

Product Details

	Box 2 (shipped on blue ice packs)
Material not included:	1. 96-well microtiter plate 2. Distilled or deionized water 3. 1X PBS 4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 6. Conical tubes and bottles for sample and buffer preparation 7. Centrifuge and/or microfuge 8. Sonicator or tissue homogenizer 9. Multichannel micropipette reservoirs 10. Ethanol 11. Microplate reader capable of reading 405 nm

Target Details

Background:	Oxidative stress occurs when there is an excess of free radicals, or reactive oxygen species (ROS), in the body. Research has shown that excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. ROS damage has been implicated in the development of many physiological problems, such as ageing, asthma, arthritis, diabetes, cancer, inflammation, cardiovascular disease, atherosclerosis, Down's Syndrome, and neurodegenerative diseases. Glutathione is a key intracellular tripeptide thiol composed of glutamic acid, cysteine, and glycine. Glutathione helps protect cells from free radical damage by acting as an antioxidant. Within cells, glutathione exists in reduced (GSH) and oxidized (GSSG) states. In healthy cells and tissue, more than 90 % of the total glutathione pool is in the reduced form (GSH) while less than 10 % exists in the disulfide form (GSSG). The high GSH concentration is because the enzyme that transitions it from its oxidized state (GSSG), glutathione reductase, is constitutively active and inducible upon oxidative stress. An increased GSSG-to-GSH ratio is considered indicative of oxidative stress. Reduced glutathione's thiol group provides reducing equivalents to other unstable ROS, which in turn becomes unstable itself. This unstable GSH readily reacts with another unstable GSH to form a stable GSSG molecule. This reaction is prevalent since glutathione is present in high concentrations. GSSG is subsequently converted to GSH again by the enzyme glutathione reductase. In addition to its role in oxidative stress, glutathione also helps maintain exogenous antioxidants such as vitamins C and E. Glutathione is involved with the breakdown of peroxides. It has a role in regulating the nitric acid cycle. Glutathione can directly bind many inorganic and organic xenobiotic (foreign chemicals) and carcinogenic compounds, such as the heavy metals mercury and arsenic. It is important to the proper function and maximum effect of the immune system. In addition, glutathione is fundamentally involved with many metabolic and

Target Details

	biochemical mechanisms such as protein and prostaglandin synthesis, DNA synthesis and repair, maintenance of disulfie bonds in proteins, enzyme activation and amino acid transport across cell membranes.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Measures total glutathione (oxidized and reduced) • Sensitive detection as low as 4 nM • Suitable for use with serum, plasma, saliva, urine, tissue extracts, and mammalian or plant cell lysates
Protocol:	Glutathione Reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. Subsequently, the chromogen reacts with the thiol group of GSH to produce a colored compound that absorbs at 405 nm . The total glutathione content in unknown samples is determined by comparison with the predetermined glutathione standard curve. The rate of chromophore production is proportional to the concentration of glutathione within the sample. The rate can be determined from the absorbance change over time. Metaphosphoric acid is provided to remove interfering proteins or enzymes from samples.
Reagent Preparation:	• 1X Assay Buffer: Prepare 1X Assay Buffer by adding 200 mL of deionized water to 50 mL of the 5X Assay Buffer. Mix thoroughly until homogeneous. Use this buffer for preparing kit reagents. Store at 4 °C when not in use. • 1X Glutathione Reductase: Prepare the 1X Glutathione Reductase by diluting the stock solution 1:50 with 1X Assay Buffer. Vortex the stock tube thoroughly prior to preparing. Prepare only enough for immediate applications (eg. Add 25 µL of Glutathione Reductase stock to 1.225 mL Assay Buffer). • 1X Chromogen: Prepare the 1X Chromogen just before use. Prepare only enough for immediate applications. Dilute the Chromogen stock 1:15 with 1X Assay Diluent (eg. Add 167 µL of Chromogen stock to 2.333 mL of 1X Assay Buffer. Vortex thoroughly. • 1X NADPH: Prepare 1X NADPH by diluting the stock solution 1:50 with 1X Assay Buffer. Vortex the stock tube thoroughly prior to preparing. Prepare only enough for immediate applications (eg. Add 25 µL of NADPH stock to 1.225 mL Assay Buffer). • Metaphosphoric Acid (MPA): Prepare a 5 % (w/v) Metaphosphoric Acid (MPA) solution in deionized water. Prepare just before use. Prepare only enough for immediate applications (eg. Add 0.5 g of Metaphosphoric Acid crystals to 10 mL of deionized water). Vortex thoroughly. Note: MPA is corrosive and may cause burns. Use caution when handling acidic reagents.
Sample Preparation:	These preparation protocols are intended as a guide for preparing known samples. The user may need to adjust how they treat their sample accordingly. All samples should be assayed

immediately or store at -80 °C for up to 1-2 months. A trial assay with a representative test sample should be assayed to determine the samples compatibility with the dynamic range of the standard. It is recommended 4 that samples be processed as soon as possible because GSH is rapidly metabolized and will continue to form various disulfides. The assay can be used on cell culture supernatants and lysates, blood, plasma, urine, saliva, tissue homogenates as well as other biological fluids. All samples should be treated with 5 % MPA to remove interfering proteins and enzymes. The MPA treated deproteinated samples improve the stability of GSH. High levels of interfering substances may cause variations in results. Run proper controls as necessary. Always run a standard curve with samples. Notes:

- Thiol compounds, such as cysteine, dithiothreitol (DTT), or β-mercaptoethanol can interfere with the assay by competing with GSH for binding to the Chromogen. In addition, N-ethylmaleimide or other thiol alkylating reagents should also be avoided because they will interfere with Glutathione Reductase and GSH.
- 5 % MPA will interfere with the assay. Upon preparing samples, each sample must be diluted 1:10 with 1X Assay Buffer to bring the MPA concentration to 0.5 % final. Some samples may need to be diluted more. Make serial dilutions of samples as necessary to obtain a quantifiable change in absorbance readings over time.
- A kinetic assay is recommended because it is more precise than an end point assay.
- Saliva, Plasma or Urine: GSSG in normal resting saliva, plasma and urine is at or below the detection limit for most glutathione assays. Collect sample in a microfuge tube and immediately add 4 volumes of ice-cold 5 % MPA. Mix thoroughly and store on ice for 15 minutes. Centrifuge at 12,000 rpm for 10 minutes at 4 °C to remove insoluble particles. Collect the supernatant. Store on ice if assaying immediately or freeze at -80 °C for future use.
- Cell Lysate: Detach adherent cells by trypsinization. Count cells and centrifuge at 500 rpm for 5 minutes at 4 °C. Wash cells with cold 1X PBS. Centrifuge suspension cells at 500 rpm for 5 minutes at 4 °C. Remove supernatant and wash cells with cold 1X PBS. Repeat centrifugation and remove solution. Immediately resuspend the pellet with 200-500 µL ice-cold 5 % MPA for a cell concentration of 1-5 x 106 cells. Mix thoroughly. Homogenize or sonicate cell suspension and store on ice until use. Transfer the suspension to a microfuge tube and centrifuge at 12,000 rpm for 5 minutes at 4 °C. Collect the supernatant. Store on ice if used immediately or freeze at - 80 °C for future use.
- Tissue Lysate: The GSH concentration in most tissue is in the 1-10 mM range. It is recommended that a 10 % w/v homogenate be created. Blood can contaminate a tissue sample due to high GSH concentrations. Therefore, perfusion of the tissue with a PBS/heparin is recommended. Remove tissue and wash the tissue thoroughly with cold isotonic saline solution of 1X PBS with 0.16 mg/mL heparin to prevent coagulation. Blot the tissue dry and weigh. Add ice-cold 5 % MPA (~1 mL/100 mg tissue) and homogenize using a glass pestle. Centrifuge the homogenate at 12,000 rpm for 15 minutes at 4 °C. Collect the supernatant. Store on ice if used immediately or freeze at -80 °C for future use.
- Erythrocyte Lysate: Add blood sample to a blood collection tube with an anticoagulant such as heparin or sodium citrate. Centrifuge at 3,000 rpm for 15 minutes at 4 °C. Remove and

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discard any plasma supernatant. Remove the white buffy coat (leukocytes) on the surface of the erythrocytes. Add four volumes of ice-cold 5 % MPA to the pellet and resuspend. Mix thoroughly. Store on ice for 10 minutes. Centrifuge the suspension at 12,000 rpm for 10 minutes at 4 °C. Collect the supernatant. Store on ice if used immediately or freeze at -80 °C for future use. 5

- Whole Blood Lysate: Collect blood in conical tubes containing an anticoagulant such as sodium citrate or heparin. Add four volumes of ice-cold 5 % MPA and mix thoroughly. Store on ice for 10 minutes. Centrifuge the suspension at 12,000 rpm for 10 minutes at 4 °C. Collect the supernatant. Store on ice if used immediately or freeze at -80 °C for future use.

Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-80 °C
Storage Comment:	Upon receipt, store the NADPH at -80°C. Prepare single use aliquots and avoid multiple freeze/thaw cycles. Store the remaining kit components at 4°C.

Publications

Product cited in:

Avalos, Haza, Mateo, Morales: "Interactions of manufactured silver nanoparticles of different sizes with normal human dermal fibroblasts." in: **International wound journal**, Vol. 13, Issue 1, pp. 101-9, (2016) (PubMed).

Song, Seo, Kim, Moon, Won, Son, Son, Kwon: "Protective Effects of Manassantin A against Ethanol-Induced Gastric Injury in Rats." in: **Biological & pharmaceutical bulletin**, Vol. 39, Issue 2 , pp. 221-9, (2016) (PubMed).

Iqbal, Riaz, Andrabi, Shahzad, Durrani, Ahmad: "l-Cysteine improves antioxidant enzyme activity, post-thaw quality and fertility of Nili-Ravi buffalo (Bubalus bubalis) bull spermatozoa." in: **Andrologia**, (2016) (PubMed).

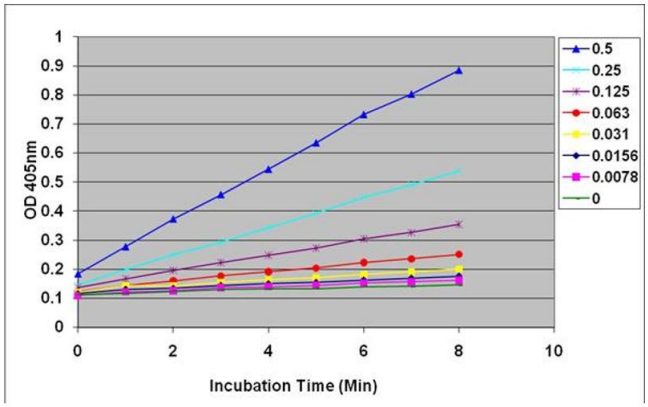
Pelster, Giacomin, Wood, Val: "Improved ROS defense in the swimbladder of a facultative air-breathing erythrinid fish, jeju, compared to a non-air-breathing close relative, traira." in: **Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology**, (2016) (PubMed).

Publications

Patel, Lawrence, Peteroy-Kelly: "Persistent Mycobacterium bovis-BCG is resistant to glutathione induced reductive stress killing." in: **Microbial pathogenesis**, Vol. 95, pp. 124-32, (2016) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1. GSSG Standard Curve. OD 405nm versus incubation time as a function of GSSG concentration.