Datasheet for ABIN577648

Glutathione Colorimetric Detection Kit

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Overview

Quantity:	4 x 96 tests
Target:	Glutathione
Reactivity:	Various Species, Human
Minimum Detection Limit:	1.78 µM
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The DetectX® Glutathione kit is designed to quantitatively measure glutathione (GSH), and oxidized glutathione (GSSG) present in a variety of samples. No separation or washing is required.
Brand:	DetectX®
Sample Type:	Blood, Serum, Plasma, Erythrocyte Lysates, Urine, Cell Lysate, Tissue Samples
Detection Method:	Colorimetric
Specificity:	Sample Types validated: Whole Blood, Serum, Plasma,Erythrocytes, Urine, Cell Lysates and Tissue Samples
Sensitivity:	0.634 µM
Characteristics:	The Glutathione (GSH) kit utilizes a colorimetric substrate that reacts with the free thiol group on GSH to yield a highly colored product. All reagents are in solution and require simple dilution for use in the assay. Free GSH concentration in the sample is calculated from the difference between the total GSH determined and the GSH generated from GSSG. The concentration of GSH can be determined either as an endpoint read of the color developed at 405nm after 30

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	minutes or by measuring the rate of color development at 405nm. Glutathione (GSH) is the highest concentration non-protein thiol in mammalian cells and is present in concentrations of 0.5 to 10 mM. GSH plays a key role in many biological processes, including the synthesis of proteins and DNA, the transport of amino acids, and the protection of cells against oxidation. Harmful hydrogen peroxide cellular levels are minimized by the enzyme glutathione peroxidase (GP) using GSH as a reductant. The oxidized GSH dimer, GSSG, is formed from GSH and peroxide by the GP reaction. An important role of GSSG in the NFkB activating signal cascade is suggested by the fact that the potent NFkB inducer TPA increases intracellular GSSG levels and GSSG/GSH ratios. [33]
Components:	Clear 96 Well Plate 4 Plates Oxidized Glutathione Standard Oxidized Glutathione at 250 µM in a special stabilizing solution. 350 µL Detection reagent Concentrate Detection substrate in DMSO. 1 mL Assay Buffer A phosphate buffer containing chelators and stabilizers. 225 mL NADPH Concentrate Reduced ß-nicotinamide adenine dinucleotide 2'-phosphate (NADPH) as a stable solution. 1 mL Glutathione reductase Conc. Glutathione Reductase (GR) as a stable solution. 1 mL
Material not included:	Distilled or deionized water. Repeater pipet with disposable tips capable of dispensing 25 µL. Aqueous 5-sulfo-salicylic acid dihydrate (SSA) solution at 5 % weight/volume (1g of SSA per 20 mL of water) for treating samples to remove protein. We recommend Sigma-Aldrich . 2-Vinylpyridine (2VP) is used to block any free GSH or other thiols present in the treated samples. 2VP is prepared by adding 27 µL of 2-vinylpyridine (such as Sigma) to 98 µL of ethanol. Use immediately and discard remaining unused solutions. A 96 well plate reader capable of reading optical absorption at 405-412 nm. Software for converting raw optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	Glutathione
Abstract:	Glutathione Products
Target Type:	Chemical
Background:	Glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) is the highest concentration non-protein thiol

Target Details

	in mammalian cells and is present in concentrations of 0.5 - 10 mM1. GSH plays a key role in many biological processes, including the synthesis of proteins and DNA, the transport of amino acids, and the protection of cells against oxidation. Harmful hydrogen peroxide cellular levels are minimized by the enzyme glutathione peroxidase (GP) using GSH as a reductant2. Sh nh o o 2 h o n ho n oh h o The oxidized GSH dimer, GSSG, is formed from GSH and peroxide by the GP reaction (see below). An important role of GSSG in the NFKß activating signal cascade is suggested by the facts that the potent NFKß inducer, tetradecanoyl phorbol acetate, increases intracellular GSSG levels and GSSG/GSH ratios3. h 2 o h 2 o2 Glutathione Peroxidase GSSG GSh Glutathione reductase Glutathione S-transferase nADPh nADP+ GSh Conjugate Glutathione S-transferases (GST) are an important group of enzymes that catalyze the nucleophilic addition of GSH to electrophiles. They are encoded by 5 gene families, 4 encode cytosolic GST and one encodes the microsomal form of GST. They have been implicated in a number of diseases. In asthma arachidonic acid is converted to unstable leukotriene A (LTA). LTA is either hydrated 4 4 4 to form LTB or it is conjugated to GSH by a GST, leukotriene C synthase, to form leukotriene C . 4 4 4 LTC and its derivative LTD are important molecules in bronchial asthma. Leukotriene C synthase 4 4 4 is therefore an important therapeutic target. It has also been shown that increased expression of GSTs can lead to drug resistance. Three glutathione adducts of the drug melphalan, used to treat ovarian cancer and multiple myeloma, have been isolated from reactions involving human microsomal GSTs

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Application Notes:	GSH is identical across species and we expect this kit may measure GSH from sources other than human. The end user should evaluate recoveries of GSH in samples from other species being tested. If samples need to be stored after collection, we recommend storing them at -70 °C or lower, pref- erably after being frozen in liquid nitrogen. This assay has been validated for human whole blood, serum, EDTA and heparin plasma, urine, and isolated erythrocytes. Most cell lysates and tissue ho- mogenates should also be compatible. Samples containing visible particulate should be centrifuged prior to using. All samples will be deproteinized with 5 % SSA (see page 6 for preparation), please see sample specific information below for details. This treatment removes any protein thiols present in the samples and also slows oxidation of free GSH.
Assay Time:	1 h

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Protocol:	A GSSG standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a colorimetric substrate that reacts with the free thiol group on GSH to yield a highly colored product. Supplied reagents are in solution and require simple dilution for use in the assay. By using 2-Vinylpyridine (not supplied) to block any free GSH in the sample, Oxidized Glutathione (GSSG) can be determined. Any samples that have not been treated with 2-Vinylpyridine will yield Total GSH levels. The Free GSH concentration in the sample is calculated from the difference between the Total GSH determined and the GSH generated from Oxidized Glutathione for the 2-Vinylpyridine treated samples. The concentration of GSH can be determined either as an endpoint read of the color developed at 405 nm or by measuring the rate of color development at 405 nm. Our Fluorescent Glutathione kits (Catalog Numbers K006-F1 and K006-F5) allow the measurement of both Free and Oxidized Glutathione with higher sensitivity in the same sample in the same well without using 2-Vinylpyridine.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine GSH concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Sample Diluent Prepare the Sample Diluent by diluting one part 5 % SSA 1:5 with four parts Assay Buffer and vortex thoroughly. The pH of the Sample Diluent must be > 6. Sample Diluent can be stored at 4 °C for one month. 2-Vinylpyridine treatment To measure Oxidized Glutathione, free GSH must be blocked by alkylation. To 250 µL of SSA treated samples, standards or Sample Diluent add 5 µL of the ethanolic solution of 2VP (see page 6) and allowed to incubate at room temperature for 1 hour. The 2VP treated samples and standards should then be diluted in Assay Buffer and Sample Diluent according to the dilutions recommended for each sample type on pages 7 and 8 prior to using in the assay. The 2VP treated Sample Diluent is used for the zero standard on page 11. Samples treated with 2VP should be read off a standard curve generated with 2VP treated standards. Colorimetric Detection reagent Prepare the Colorimetric Detection Reagent by diluting one part

Colorimetric Detection Reagent Concentrate 1:10 with nine parts Assay Buffer.
See Colorimetric Detection Reagent Dilution Table for suitable volumes.
Colorimetric Detection reagent Dilution table 1/2 Plate one Plate two Plates four Plates Colorimetric Detection Concentrate 140 µL 260 µL 500 µL 1 mL Assay Buffer 1.26 mL 2.34 mL 4.5 mL 9 mL total Colorimetric reagent Volume 1.4 mL 2.6 mL 5 mL 10 mL reaction Mixture
Prepare the Reaction Mixture by diluting one part each NADPH and Glutathione Reductase Concentrates 1:10 into eight parts Assay Buffer.
See Reaction Mix Dilution Table for suitable volumes.
Store any unused Reaction Mixture at 4 °C for no more than 2 days. reaction Mix Dilution table 1/2 Plate one Plate two Plates four Plates nADPh Concentrate 140 µL 260 µL 500 µL 1 mL Glutathione reductase Concentrate 140 µL 260 µL 500 µL 1 mL Assay Buffer 1.12 mL 2.08 mL 4 mL 8 mL total reaction Mix Volume 1.4 mL 2.6 mL 5 mL 10 mL ® www.ArborAssays.com 9
Standard Preparation for the measurement of oxidized Glutathione (GSSG), a 50 µL aliquot of the 250 µM Oxidized Glutathione Standard should be treated with 1 µL of 2VP as outlined on page 9. 2VP-treated Standards are prepared by labeling test tubes as #1 through #6.
Pipet 475 µL of Sample Diluent into tube #1 and 250 µL into tubes #2 to #6.
Carefully add 25 µL of the 2VP-treated Standard to tube #1 and vortex completely.
Take 250 µL of the solution in tube #1 and add it to tube #2 and vortex completely.
Repeat this for tubes #3 through #6.
The concentration of Oxidized Glutathione in tubes 1 through 6 will be 12.5, 6.25, 3.125, 1.56, 0.781 and 0.391 µM.
The concentration of Total GSH in tubes 1 through 6 will be 25, 12.5, 6.25, 3.125, 1.56, and 0.781 µM after addition of the Reaction Mixture. 2VP treated Sample Diluent must be used as a 0 µM standard. to determine total GSh, the Standards are prepared by labeling test tubes as #1 through #6.
Pipet 475 µL of Sample Diluent into tube #1 and 250 µL into tubes #2 to #6.
Carefully add 25 µL of the supplied Standard to tube #1 and vortex completely.
Take 250 µL of the solution in tube #1 and add it to tube #2 and vortex completely.
Repeat this for tubes #3 through #6.
The concentration of Total GSH in tubes 1 through 6 will be 25, 12.5, 6.25, 3.125, 1.56, and 0.781 µM after addition of the Reaction Mixture.
Sample Diluent must be used as a 0 µM standard. use all Standards within 2 hours of preparation.
Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Sample Diluent Volume (µl) 475 250 250 250 250 250 Addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Volume of Addition (µl) 25 250 250 250 250 250 GSSG Conc (µM) 12.5 6.25 3.125 1.56 0.781 0.391 total GSh Conc (µM) 25 12.5 6.25 3.125 1.56

	0.781 ® 10 EXPECT ASSAY ARTISTRY ASSAy ProtoCol - enD Point for oxidized Glutathione (GSSG) use the 2VP treated standards, 2VP treated Sample Diluent and 2VP treated samples diluted with Sample Diluent as described previously. for total Glutathione use the standards and samples diluted with Sample Diluent as described previously. 1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. 2. Pipet 50 µL of either 2VP treated or untreated samples or standards into duplicate wells in the plate. 3. Pipet 50 µL of either 2VP treated or untreated Sample Diluent into duplicate wells as the Zero standard. 4. Add 25 µL of the Colorimetric Detection Reagent to each well using a repeater pipet. 5. Add 25 µL of the Reaction Mixture to each of the wells using a repeater pipet. 6. Gently tap the sides of the plate to ensure adequate mixing of the reagents. 7. Incubate at room temperature for 20 minutes. 8. Read the optical density at 405 nm. These data will be used to determine either Oxidized Glutathione or Total Glutathione concentration. ASSAy ProtoCol - KinetiC 1. Carry out steps 1-4 above. 2. Gently tap the sides of the plate to ensure adequate mixing of the reagents. 3. Add 25 µL of the Reaction Mixture to each of the wells using a repeater and immediately place plate in reader and read optical density at 405 nm every minute for at least 10 minutes. These data will be used to determine Total or Oxidized Glutathione concentration kinetically.
Sample Preparation:	All samples must be treated with the SSA solution prepared on page 6. All of the SSA treated centrifuged supernatants must have their SSA concentration brought down to 1 % SSA by dilution with Assay Buffer. Further dilutions of the sample, using Sample Diluent (see page 9 for preparation), may be necessary to allow the GSH concentration to be measurement in the assay. Detailed instructions follow. All samples and standards must be in Sample Diluent before starting the assay To measure Oxidized Glutathione in samples, reduced Glutathione (GSH) in the sample must be blocked by treatment with 2-vinylpyridine, 2VP (see page 6 for preparation). SSA treated samples should be treated with 2VP by addition of 5 µL of 2VP solution for every 250 µL of sample (see page 9). 2VP treated samples must be read off a standard curve made with 2VP-treated standards. use all samples within 2 hours of dilution. Whole Blood, Serum, eDtA or heparin Plasma, or urine Thoroughly mix sample with an equal volume of cold 5 % SSA. Incubate for 10 minutes at 4 °C. Centrifuge at 14,000 rpm for 10

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	minutes at 4 °C. Collect the supernatant. If the supernatent contains particulates, re-centrifuge the supernatant for 15 minutes and collect the clarified second supernatant. Samples can be stored in aliquots at ≤ -70 °C or analyzed immediately. At this point the SSA concentration will be 2.5 %. The supernatant must be diluted 1:2.5 with Assay Buffer by mixing one part with 1.5 parts of Assay Buffer to bring the SSA concentration to 1 %. The sample will have been diluted 1:5 at this point. All final dilutions are made in Sample Diluent. Treated Whole Blood must be further diluted at least 1:20 for a recommended final dilution of ≥ 1:100. For Treated Plasma and Treated Urine a final dilution of ≥ 1:5 is recommended, but further dilutions in Sample Diluent may be necessary.
Calculation of Results:	Average the duplicate optical density readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean ODs for the zero standard. The concentrations obtained should be multiplied by the dilution factor to obtain sample values.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product. Sulfosalicylic acid is a strong acid solution and should be treated like any other laboratory acid. 2VP is toXiC and may cause burns. 2VP solutions should be prepared in a fume hood. Use immediately and discard remaining unused solutions by mixing with copious amounts of water. Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:	Allan, Hafez, Labrecque, Solomon, Shaikh, Zheng, Ali: "Sex-Dependent effects of developmental arsenic exposure on methylation capacity and methylation regulation of the glucocorticoid receptor system in the embryonic mouse brain." in: **Toxicology reports**, Vol. 2, pp. 1376-1390, (

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Ly, Morris, Lagman, Christopher, Anderson, Daliva, Muwanas, Tarash, Ochoa, Sathananthan, Venketaraman: "Complement 3 Receptor Expression in Individuals with Type 2 Diabetes." in: **Recent patents on anti-infective drug discovery**, (2016) (PubMed).

Peiró, Romacho, Azcutia, Villalobos, Fernández, Bolaños, Moncada, Sánchez-Ferrer: " Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway." in: **Cardiovascular diabetology**, Vol. 15, pp. 82, (2016) (PubMed).

Yu, Long: "Crosstalk between cystine and glutathione is critical for the regulation of amino acid signaling pathways and ferroptosis." in: **Scientific reports**, Vol. 6, pp. 30033, (2016) (PubMed).

There are more publications referencing this product on: Product page

Images

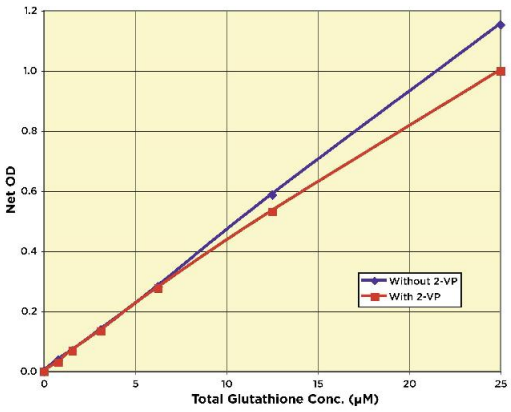

Image 1.