Datasheet for ABIN577646

# Glutathione Fluorescent Detection Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | Glutathione |
| Reactivity: | Various Species, Human |
| Minimum Detection Limit: | 42 nM |
| Application: | Biochemical Assay (BCA) |

## Product Details

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| Purpose: | The DetectX® Glutathione kit is designed to quantitatively measure glutathione (GSH), and oxidized glutathione (GSSG) present in a variety of samples. |
| Brand: | DetectX® |
| Sample Type: | Blood, Serum, Plasma, Erythrocyte Lysates, Urine, Cell Lysate, Tissue Samples |
| Detection Method: | Fluorometric |
| Specificity: | Sample Types validated: Whole Blood, Serum, Plasma, Erythrocytes, Urine, Cell Lysates and Tissue Samples |
| Sensitivity: | 45 nM in the Free GSH and 48 nM in the Total GSH assays. |
| Characteristics: | The Glutathione (GSH) Fluorometric kit utilizes a proprietary non-fluorescent molecule, ThioStar®, that covalently binds to the free thiol group on GSH to yield a highly fluorescent product. After mixing the sample or standard with ThioStar® and incubating at room temperature for 15 minutes, the GSH-generated signal is read at 510 nm in a fluorescent plate reader with excitation at 390 nm. Addition of a reaction mixture converts all the GSSG into free GSH, which then reacts with the excess ThioStar® during a second 15 minute incubation, |

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| | yielding the signal related to Total GSH content. 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. |
| Components: | Black 96 Well Plate 1 or 5 each<br>Glutathione Standard Glutathione at 250 µM in a special stabilizing solution. 100 µL or 3µL<br>ThioStar® Detection reagent ThioStar thiol detection substrate stored in a ziploc pouch with desiccant. Reconstitute with dry DMSO. 2 Plastic vials or 4 Glass vials<br>Dry DMSO Dry Dimethyl sulfoxide solvent over molecular sieves. May be stored at room temperature. 4 mL or 20 mL<br>Assay Buffer Concentrate A 2X buffer concentrate containing detergents and stabilizers that must be diluted with deionized or distilled water. 35 mL or 200 mL<br>NADHP Concentrate Reduced ß-nicotinamide adenine dinucleotide 2'-phosphate (NADPH) as a stable solution. 300 µL or 1.4 mL<br>Glutathione reductase Concentrate Glutathione Reductase (GR) as a stable solution. 300 µL or 1.4 mL<br>Oxidized Glutathione Control Oxidized Glutathione (GSSG) in a special stabilizing solution. This is an optional control solution to ensure NADPH/GR performance. 300 µL |
| Material not included: | Distilled or deionized water Repeater pipet with disposable tips capable of dispensing 25 µL.<br>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.<br>We recommend Sigma-Aldrich .<br>Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm.<br>Please contact your plate reader manufacturer for suitable filter sets.<br>Set plate parameters for a 96-well Corning Costar 3650 plate.<br>See: www.arborassays.com/ resources/#general-info for plate dimension data.<br>The sensitivity of fluorescent assays is dependant on the capabilities of the plate reader.<br>If your plate reader has adjustable gain you can modify the signals obtained from the assay by increasing or decreasing the gain settings, by changing the aperture settings for |

## Product Details

monochromator based readers, or by changing the band pass width of the emission and/or excitation filters on some readers.
Please review the plate reader manual for details. signals expressed by plate readers are relative Fluorescent units (rFu) and the values given in the insert were obtained on our plate readers. the rFu numbers you obtain may be different from these, but the assay results should be similar.
Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

## Target Details

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| Target: | Glutathione |
| Abstract: | Glutathione Products |
| Target Type: | Chemical |
| Background: | Glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) is the highest concentration non-protein thiol 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 NFKB activating signal cascade is suggested by the facts that the potent NFKB 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 |

## Application Details

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| Application Notes: | GSH is identical across species and we expect this kit may measure GSH from sources other than human.<br>The end user should evaluate recoveries of GSH in samples from other species being tested.<br>If samples need to be stored after collection, we recommend storing them at -70 °C or lower, preferably after being frozen in liquid nitrogen.<br>This assay has been validated for human whole blood, serum, EDTA and heparin plasma, urine, and isolated erythrocytes.<br>Most cell lysates and tissue homogenates should also be compatible.<br>Samples containing visible particulate should be centrifuged prior to using.<br>All samples will be deproteinized with 5 % SSA (see page 6 for preparation), please see sample specific information below for details.<br>This treatment removes any protein thiols present in the samples and also slows oxidation of free GSH. |
| Protocol: | The kit is unique in that both free and oxidized glutathione are detected in the same well in the microtiter plate.<br>No separation or washing is required.<br>Total glutathione is the sum of GSSG plus GSH.<br>Please read the complete kit insert before performing this assay.<br>A GSH standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve.<br>The kit utilizes a proprietary non- fluorescent molecule, ThioStar®, that will covalently bind to the free thiol group on GSH to yield a highly fluorescent product.<br>After mixing the sample or standard with ThioStar® and incubating at room temperature for 15 minutes, the fluorescent product is read at 510 nm in a fluorescent plate reader with excitation at 390 nm.<br>The concentration of the GSH in the sample is calculated, after making a suitable correction for any dilution of the sample, using software available with most fluorescence plate readers.<br>Free glutathione, GSH, is read first after 15 minutes, followed by addition of a reaction mixture that converts all the oxidized glutathione, GSSG, into free GSH, which then reacts with the excess ThioStar® to yield the signal related to Total GSH content.<br>The total concentration of GSH generated in the sample is calculated from the generated signal.<br>We have provided a 96 well plate for measurement but this assay is adaptable for higher density plate formats.<br>The end user should ensure that their HTS black plate is suitable for use with these reagents |

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| | prior to running samples. |
| Reagent Preparation: | Allow the kit reagents to come to room temperature for 30 minutes.<br>Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. assay Buffer Prepare the Assay Buffer by diluting the supplied Assay Buffer Concentrate with an equal volume of deionized water.<br>Mix thoroughly.<br>Stable at 4 °C for 3 months.<br>Sample Diluent Prepare the Sample Diluent by diluting one part 5 % SSA 1:5 with four parts diluted Assay Buffer and vortex thoroughly.<br>The pH of the Sample Diluent must be > 6.<br>Sample Diluent can be stored at 4 °C for one month.<br>Standard Preparation GSH Standards are prepared by labeling eight test tubes as #1 through #8.<br>Briefly vortex to mix and then spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial.<br>Pipet 450 µL of Sample Diluent into tube #1 and 250 µL into tubes #2 to #8.<br>Carefully add 50 µL of the Glutathione Standard to tube #1 and vortex completely.<br>Take 250 µL of the GSH solution in tube #1 and add it to tube #2 and vortex completely.<br>Repeat this for tubes #3 through #8.<br>The concentration of GSH in tubes 1 through 8 will be 25, 12.5, 6.25, 3.125, 1.56, 0.781, 0.391 and 0.195 µM.<br>Use all Standards within 1 hour of preparation.<br>Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Std 7 Std 8 Sample Diluent Vol (µL) 450 250 250 250 250 250 250 250 addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Std 7 Vol of addition (µL) 50 250 250 250 250 250 250 250 Final Conc (µM) 25 12.5 6.25 3.125 1.56 0.781 0.391 0.195 ® www.ArborAssays.com 9 Control Preparation (optional) This optional control solution for ensuring complete conversion of GSSG to GSH can be prepared by adding 5 µL of Oxidized Glutathione Control to 245 µL of Sample Diluent. use within 2 hours.<br>The Control Preparation ensures that the NADPH and Glutathione Reductase system prepared below in the Reaction Mixture section will adequately reduce GSSG to GSH.<br>If this optional control is run it should yield a value for Total Glutathione of approximately 10 ± 2 µM. thioStar® Detection reagent Allow the ziploc bag to warm completely to room temperature prior to opening and remove the vial of ThioStar Reagent.<br>Add the volume of DMSO provided to the vial according to the table below.<br>Vortex thoroughly. |

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| | Store any unused reconstituted Detection Reagent at 4 °C in the ziploc pouch with desiccant and use within 2 months.<br>Kit K006-F1 Kit K006-F5 Vial Part Number C021-1EA, Plastic vial C036-1EA, Glass vial Volume of DMSO to add per vial 1.5 mL 3.5 mL For # of Wells Up to 60 Up to 140 reaction Mixture Prepare the Reaction Mixture by vortexing the vials of Glutathione Reductase and NADPH Concentrates and then diluting one part each NADPH and Glutathione Reductase Concentrates 1:10 into eight parts Assay Buffer.<br>Vortex thoroughly.<br>See Table for suitable volumes.<br>Store any unused Reaction Mixture at 4 °C in an amber vial for no more than 2 days. reaction Mix Dilution table 1/2 Plate one Plate NADPH Concentrate 150 µL 275 µL Glutathione Reductase Concentrate 150 µL 275 µL Assay Buffer 1.2 mL 2.2 mL ® 10 EXPECT ASSAY ARTISTRY ASSAy ProtoCol - Free AnD totAl GSh We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine GSh concentrations. 1.<br>Use the plate layout sheet on the back page to aid in proper sample and standard identification.<br><br>Set plate parameters for a 96-well Corning Costar 3650 plate. 2.<br>Pipet 50 µL of treated samples, standards or control into wells in the plate. 3.<br>Pipet 50 µL of Sample Diluent into Zero wells in the plate. 4.<br>Add 25 µL of the ThioStar Reagent to each well using a repeater pipet. 5.<br>Gently tap the sides of the plate to ensure adequate mixing of the reagents. 6.<br>Incubate at room temperature for 15 minutes. 7.<br>Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent emission at 510 nm with excitation at 370-410 nm.<br>This data will be used to determine Free GSH concentration. 8.<br>Add 25 µL of the Reaction Mixture to each of the wells using a repeater pipet. 9.<br>Gently tap the sides of the plate to ensure adequate mixing of the reagents. 10.<br>Incubate at room temperature for 15 minutes. 11.<br>Read the fluorescent emission at 510 nm with excitation at 370-410 nm.<br>This data will be used to determine Total GSH concentration. total GSh Content only Total GSH content can be determined directly by leaving out steps 5, 6 and 7. |
| 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 |

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| | 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. 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 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. The SSA concentration will be 1 %. The sample will have been diluted 1:5 at this point. All final dilutions are to be 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 FLU readings for each standard and sample.<br>Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard.<br>The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values. |
| Restrictions: | For Research Use only |

## Handling

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| Precaution of Use: | As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction.<br>The complete insert should be read and understood before attempting to use the product.<br>Sulfosalicylic acid is a strong acid solution and should be treated like any other laboratory acid.<br>Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances.<br>Wear protective gloves when using the solvent especially when it contains dissolved chemicals.<br><br>NOTE: DMSO can dissolve certain plastics used in troughs used for holding solutions for multichannel pipets. thiostar® thiol detection reagent should be stored at 4°c in the desiccated pouch. allow desiccated pouch to warm to room temperature prior to opening. thiostar will react with strong nucleophiles.<br>Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate. |

## Handling

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| | Reconstituted ThioStar in DMSO stored at 4°C in the desiccated pouch.<br>It can be used up to 2 months later.<br>The background on the reconstituted ThioStar will increase slowly over time but the increase will not affect the assay results obtained. |
| Storage: | 4 °C |
| Storage Comment: | All components of this kit should be stored at 4°C until the expiration date of the kit. DMSO, when stored at 4°C, will freeze. Can be stored tightly capped at room temperature. |

## Publications

Product cited in:

McAllister, Basham, Waldman, Smith, Mettler, Butawan, Bloomer: "Effects of psychological stress during exercise on markers of oxidative stress in young healthy, trained men." in: **Physiology & behavior**, Vol. 198, pp. 90-95, (2019) (PubMed).

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Rainio, Margus, Lehmann, Helander, Lindström: "Effects of a glyphosate-based herbicide on survival and oxidative status of a non-target herbivore, the Colorado potato beetle (Leptinotarsa decemlineata)." in: **Comparative biochemistry and physiology. Toxicology & pharmacology : CBP**, Vol. 215, pp. 47-55, (2018) (PubMed).

Singh, Kim, Kim: "Methionine sulfoxide reductase A deficiency exacerbates acute liver injury induced by acetaminophen." in: **Biochemical and biophysical research communications**, Vol. 484, Issue 1, pp. 189-194, (2017) (PubMed).

Kim, Kwak, Singh, Gladyshev, Kim: "Selenoprotein MsrB1 deficiency exacerbates acetaminophen-induced hepatotoxicity via increased oxidative damage." in: **Archives of biochemistry and biophysics**, Vol. 634, pp. 69-75, (2017) (PubMed).

There are more publications referencing this product on: Product page

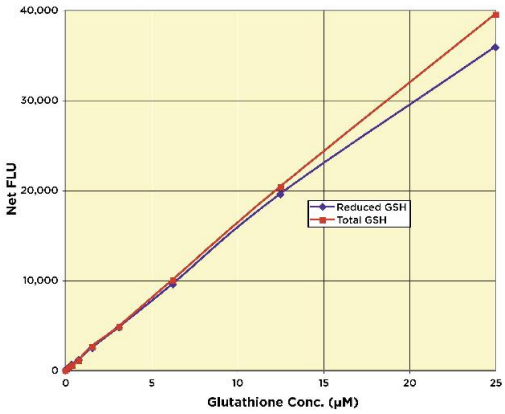
40,000
30,000
20,000
10,000
0
Net FLU
0
5
10
15
20
25
Glutathione Conc. (µM)
Reduced GSH
Total GSH

**Image 1.**