ARBOR ASSAYS

DetectX®

Glutathione
384-Well Plate
Fluorescent Detection Kit

Two 384-Well Plate Kit **Catalog Number K006-F1D**

Sample Types Validated:

Whole Blood, Serum, Plasma, Erythrocytes, Urine, Cell Lysates and Tissue Samples

Please read this insert completely prior to using the product.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

www.ArborAssays.com

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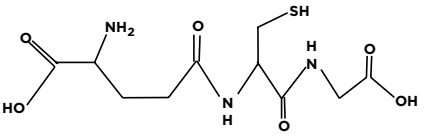
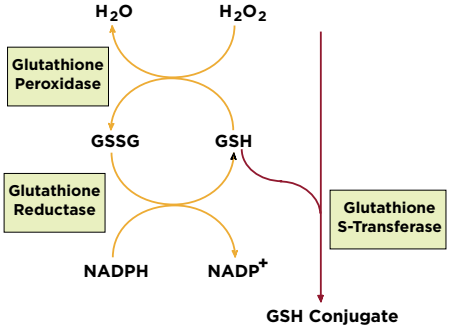
BACKGROUND

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 mM[1]. 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[2].



The oxidized GSH dimer, GSSG, is formed from GSH and peroxide by the GP reaction (see below). An important role of GSSG in the NF*K*B activating signal cascade is suggested by the facts that the potent NF*K*B inducer, tetradecanoyl phorbol acetate, increases intracellular GSSG levels and GSSG/GSH ratios[3].



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_4 (LTA_4). LTA_4 is either hydrated to form LTB_4 or it is conjugated to GSH by a GST, leukotriene C_4 synthase, to form leukotriene C_4. LTC_4 and its derivative LTD_4 are important molecules in bronchial asthma. Leukotriene C_4 synthase 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.

1. Meister, A. "On the Discovery of Glutathione." Trends Biochem. Sci. 1988 13(5): 185-188.

2. Meister, A. "The Glutathione-Ascorbic Acid Antioxidant Systems in Animals" J. Biol. Chem. 1994 269:9397-9400.

3. Dröge W, et al., "Functions of Glutathione and Glutathione Disulfide in Immunology and Immunopathology" FASEB J., 1994 8:1131-1138.

Assay Principle

The DetectX® Glutathione kit is designed to quantitatively measure glutathione (GSH), and oxidized glutathione (GSSG) present in a variety of samples. The kit is unique in that free and oxidized glutathione are detected in a 384-well microtiter plate. No separation or washing is required. Total glutathione is the sum of GSSG plus GSH. Please read the complete kit insert before performing this assay. GSH and GSSG standards are provided to generate standard curves for the assay and all samples should be read off the standard curve. 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. 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. 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.

Oxidized glutathione, GSSG, is measured after blocking any GSH in the sample by treatment with 2-Vinylpyridine. GSSG is converted into free GSH using our stable, liquid formulations of NADPH and Glutathione Reductase. The GSH formed then reacts with ThioStar® to yield the signal related to Oxidized GSH content. The total concentration of GSH generated in the sample is calculated from the measured Reduced and Oxidized GSH. We have provided special 384 well plates for measurement but this assay is adaptable for higher density plate formats. The end user should ensure that their HTS black plate is suitable for use with these reagents prior to running samples.

Related Products

KITS

Glutathione Colorimetric Detection Kit	Catalog Number K006-H1
Glutathione Fluorescent Detection Kits	Catalog Number K006-F1/-F5
Glutathione S-Transferase Fluorescent Activity Kit	Catalog Number K008-F1
Glutathione Reductase Fluorescent Activity Kit	Catalog Number K009-F1

REAGENTS

Glutathione Mouse Monoclonal Antibody, 50 µg — Catalog Number A001-50UG
Mouse IgG_{2a}, Clone L4H raised to glutathione conjugated to KLH
Applications: Western blotting, Immunoassay and Immunoprecipitation

DyLight® 488 Glutathione Mouse Monoclonal Antibody, 50 µg — Catalog Number A001F-50UG
Purified monoclonal labeled with a stable FITC like fluorescent dye
Applications: Flow cytometry and direct immunofluorescence

Supplied Components

Black 384 Well Plate 2 Each Catalog Number X115-2EA
See: http://www.ArborAssays.com/resources/lit.asp for plate dimension data.

Reduced Glutathione Standard 100 µL Catalog Number C018-100UL
Glutathione at 250 µM in a special stabilizing solution.

Oxidized Glutathione Standard 350 µL Catalog Number C020-350UL
Oxidized Glutathione at 250 µM in a special stabilizing solution

ThioStar® Detection Reagent 1 Bottle Catalog Number C036-1EA
ThioStar thiol detection substrate stored in a desiccator. Reconstitute with dry DMSO.

Dry DMSO 5 mL Catalog Number X022-5ML
Dry Dimethyl sulfoxide solvent over molecular sieves. May be stored at room temperature.

Assay Buffer Concentrate 200 mL Catalog Number X051-200ML
A buffer containing detergents and stabilizers. A 2X concentrate that should be diluted with deionized or distilled water.

NADPH Concentrate 500 µL Catalog Number X044-500UL
Reduced ß-nicotinamide adenine dinucleotide 2'-phosphate (NADPH) as a stable solution.

Glutathione Reductase Concentrate 500 µL Catalog Number X048-500UL
Glutathione Reductase (GR) as a stable solution.

Storage Instructions

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.

Other Materials Required

Distilled or deionized water

Repeater pipet with disposable tips capable of dispensing 5 µL.

Aqueous 5-sulfo-salicylic acid dihydrate (SSA, Sigma-Aldrich Catalog Number S2130) solution at 5% weight/volume (1g of SSA per 20 mL of water) for treating samples to remove protein.

2-vinylpyridine (Sigma Catalog Number 132292) and ethanol (Sigma Catalog Number 459828).

Fluorescence 384 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm. Please contact your plate reader manufacturer for suitable filter sets. Set plate parameters for a 384-well Corning Costar 3676 plate. See: http://www.ArborAssays.com/resources/lit.asp for plate dimension data.

The sensitivity of fluorescent assays is dependant on the capabilities of the plate reader. 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 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. Contact your plate reader manufacturer for details.

Precautions

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. 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 desiccator. Allow desiccator to warm to room temperature prior to opening. ThioStar will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate.

Sample Types

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, preferably 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 homogenates 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.

Sample Preparation

Reduced and Oxidized Glutathione Measurements

Reduced Glutathione is measured by taking the solutions prepared and diluted in Sample Diluent without any further treatment.

To measure **Oxidized Glutathione** in samples, reduced Glutathione (GSH) in the sample must be blocked by treatment with 2-vinylpyridine, (2VP). A solution of 27 µL of 2VP is added to 98 µL of ethanol in a fume hood. SSA treated samples are then treated with 1 µL of the ethanolic 2VP solution for every 50 µL of sample and incubating for 1 hour at room temperature. 2VP treated samples must be read off a standard curve made with 2VP-treated standards. **Use all samples within 2 hours of dilution.**

All samples and standards must be in Sample Diluent before starting the assay.
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.

Whole Blood, 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.

Tissue Samples

Fresh tissue is washed with ice cold PBS to remove blood then blotted on filter paper before recording wet weight. **NOTE: Samples that have been frozen will contain lysed cells. The PBS wash may contain substantial amounts of GSH and/or GSSG.**

• For Samples Where a Protein Determination is to be Obtained: Homogenize at 10 mg/250 µL in ice cold 100mM phosphate buffer, pH 7. Centrifuge at 14,000 rpm for 10 minutes at 4°C and remove an aliquot of the supernatant for protein determination. Thoroughly mix a second aliquot of the supernatant 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 to remove precipitated protein. Collect the supernatant. 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%.

• For Samples Not Requiring a Protein Determination: Homogenize at 10 mg/250 µL in ice cold 5% SSA, incubate at 10 minutes at 4°C, then centrifuge at 14,000 rpm for 10 minutes at 4°C to remove precipitated protein. Collect the supernatant. The supernatant must be diluted 1:5 with Assay Buffer by mixing one part with 4 parts of Assay Buffer. The SSA concentration will be 1%.

Further sample dilutions must be determined by the end-user since it will be dependent upon the tissue type and the amount of tissue used. These dilutions must be made in the prepared Sample Diluent.

Erythrocytes, Red Blood Cells (RBC's)

Collect blood with heparin or EDTA. Centrifuge the sample, remove and discard the plasma and white cell layer. Wash the RBC's 2 times by suspending in 3 volumes of isotonic saline (0.9%), centrifuging at 600 x g for 10 minutes and discarding the saline wash.

After the 2 washes, mix 250µL RBC's with 1mL 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. At this point the SSA concentration will be 4%. The supernatant must be diluted 1:4 with Assay Buffer by mixing one part with 3 parts of Assay Buffer. The SSA concentration will now be 1%. The sample will have been diluted 1:20 at this point. Further dilutions are made in Sample Diluent. NOTE: Human RBC's require a final dilution of 1:100-1:200 to read within the standard curve.

Cell Lysates

Washed cell pellets are resuspended at 1-10x10^6 cells/mL in cold 5% SSA (we used Jurkats at 5x10^6 cells/mL) and are lysed and deproteinized by vigorous vortexing, freeze/thaw cycling or other suitable disruption method. Incubate cells at 4°C for 10 minutes followed by centrifugation for 10 minutes at 14,000 rpm and 4°C. **NOTE: Samples that have been frozen will contain lysed cells. The PBS wash may contain substantial amounts of GSH and/or GSSG.**

The centrifuged supernatants must be diluted 1:5 with Assay Buffer by mixing one part with 4 parts of Assay Buffer. The SSA concentration will be 1%. The sample will have been diluted 1:5 at this point. Further sample dilutions must be done in Sample Diluent and need to be determined by the end-user since it will be dependent upon the cell type and number of cells used. The recommended final dilution is ≥ 1:20.

Use all samples within 2 hours of dilution.

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.

Assay Buffer

Prepare the Assay Buffer by diluting the supplied Assay Buffer Concentrate with an equal volume of deionized water. Mix thoroughly. Stable at 4°C for 3 months.

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 50 µL of SSA treated samples, standards or Sample Diluent add 1 µL of the ethanolic solution of 2VP (see page 6) and allow to incubate at room temperature for 1 hour. The 2VP treated samples 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 Oxidized Glutathione standard**.

ThioStar® Detection Reagent

Allow the desiccator to warm to room temperature prior to opening and remove the vial of ThioStar Reagent. Add 4 mL of DMSO provided to the vial. Vortex thoroughly. This volume of ThioStar Reagent is sufficient for 2 plates. Store any unused reconstituted Detection Reagent at 4°C in the desiccator and use within 2 months.

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. Vortex thoroughly. See Table for suitable volumes. 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	1 Plate
NADPH Concentrate	120 µL	225 µL
Glutathione Reductase Concentrate	120 µL	225 µL
Assay Buffer	960 µL	1.8 mL

Reduced GSH Standard Preparation

GSH Standards are prepared by labeling eight test tubes as #1 through #8. Briefly vortex to mix and then spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 90 µL of Sample Diluent into tube #1 and 50 µL into tubes #2 to #8. Carefully add 10 µL of the Glutathione Standard to tube #1 and vortex completely. Take 50 µL of the GSH solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #8. 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.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Sample Diluent Vol (µL)	**90**	50	50	50	50	50	50	50
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	50	50	50	50	50	50	50
Final GSH Conc (µM)	25	12.5	6.25	3.125	1.56	0.781	0.391	0.195

Oxidized GSSG 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 six test tubes as #1 through #6. Pipet 95 µL of Sample Diluent into tube #1 and 50 µL into tubes #2 to #6. Carefully add 5 µL of the 2VP-treated Standard to tube #1 and vortex completely. Take 50 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 Reduced 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.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Sample Diluent Volume (µL)	**95**	50	50	50	50	50
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Volume of Addition (µL)	**5**	50	50	50	50	50
GSSG Conc (µM)	12.5	6.25	3.125	1.56	0.781	0.391
Final GSH Conc (µM)	25	12.5	6.25	3.125	1.56	0.781

Use all Standards within 1 hour of preparation.

Assay Protocol - Free GSH

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3650 plate. See: http://www.ArborAssays.com/resources/lit.asp for plate dimension data.
2. Pipet 10 µL of treated samples, standards or control into wells in the plate.
3. Pipet 10 µL of Sample Diluent into Zero wells in the plate.
4. Add 5 µL of the ThioStar Reagent to each well using a repeater pipet.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 15 minutes.
7. 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. This data will be used to determine Free GSH concentration.

Assay Protocol - Oxidized GSH

1. Pipet 10 µL of 2VP-treated samples, standards or control into wells in the plate.
2. Pipet 10 µL of 2VP-treated Sample Diluent into Zero wells in the plate.
3. Add 5 µL of the ThioStar Reagent to each well using a repeater pipet.
4. Add 5 µL of the Reaction Mixture to each of the wells using a repeater pipet.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 15 minutes.
7. Read the fluorescent emission at 510 nm with excitation at 370-410 nm. This data will be used to determine Total GSH concentration.

Calculation of Results

Average the duplicate FLU 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 FLUs for the zero standard. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Total and Oxidized GSH Calculations

Free glutathione (GSH) concentrations are calculated from the data obtained from the Free GSH standard curve utilizing the curve fitting routine supplied with the plate reader.

Oxidized glutathione (GSSG) concentrations are calculated from the data obtained from the Oxidized GSH (GSSG) standard curve utilizing the curve fitting routine supplied with the plate reader. The results are expressed as **Reduced Glutathione (GSH).**

Total glutathione concentrations of the samples are calculated from the data obtained by combining the Free GSH and Oxidized Glutathione, expressed as **Reduced Glutathione (GSH)** concentrations.

Oxidized glutathione (GSSG) concentrations are obtained by dividing the measured Oxidized Glutathione, expressed as **Reduced Glutathione (GSH),** concentrations by 2. See Below:

$$\text{GSSG} = \frac{\text{Measured 2VP-Treated GSH Concentration}}{2}$$

Typical Data - Free GSH

Sample	Mean FLU	Net FLU	GSH Conc. (µM)
Zero	466	0	0
Standard 1	39,471	39,005	25
Standard 2	19,258	18,792	12.5
Standard 3	9,737	9,271	6.25
Standard 4	4,830	4,364	3.125
Standard 5	2,501	2,035	1.56
Standard 6	1,436	970	0.781
Standard 7	930	464	0.391
Standard 8	658	192	0.195
Sample 1	15,084	14,618	9.79
Sample 2	7,258	6,792	4.72
Sample 3	11,563	11,097	7.53
Sample 4	2,214	1,748	1.32

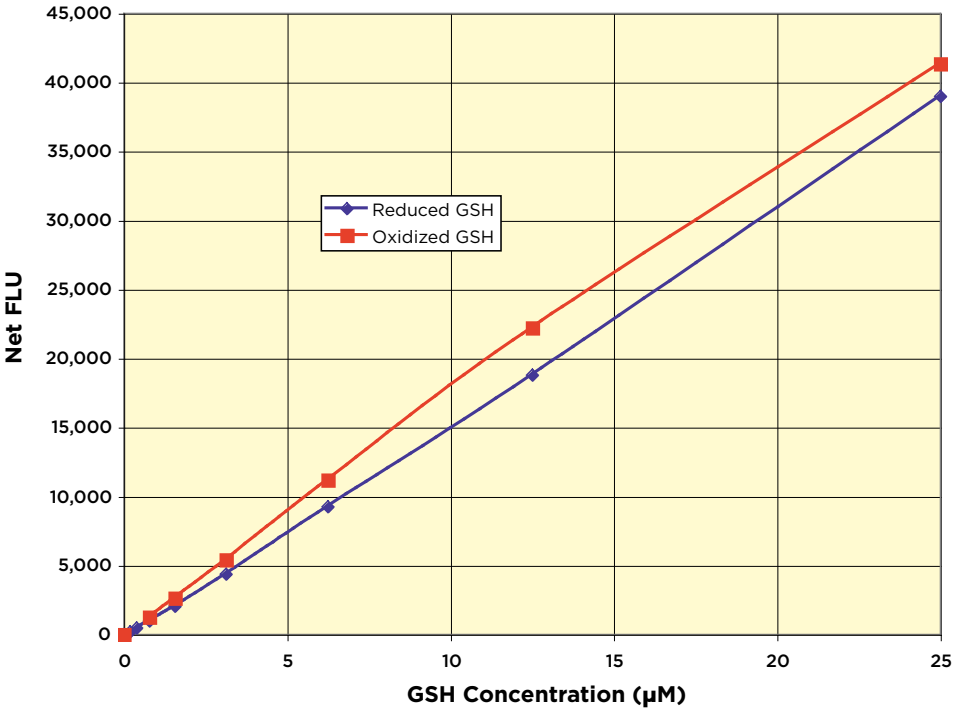
Always run your own standard curve for calculation of results.
Do not use this data.

Typical Data - Oxidized GSH

Sample	Mean FLU	Net FLU	GSH Conc. (µM)	GSSG Conc. (µM)
Zero	1,428	0	0	0
Standard 1	42,777	41,350	25	12.5
Standard 2	23,642	22,215	12.5	6.25
Standard 3	12,612	11,185	6.25	3.125
Standard 4	6,845	5,417	3.125	1.56
Standard 5	4,070	2,643	1.56	0.781
Standard 6	2,368	1,264	0.781	0.391
Sample 1	14,938	13,510	7.53	3.77
Sample 2	10,016	8,588	4.84	2.42
Sample 3	4,987	3,559	2.09	1.05
Sample 4	2,768	1,340	0.82	0.41

Always run your own standard curve for calculation of results.
Do not use this data.

Typical Standard Curves



Always run your own standard curves for calculation of results.
Do not use these data.

Data Presented Below was Obtained with our 96-well Fluorescent Glutathione Kit, K006-F1/F5, and is expected to be similar.

Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and standard #8. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 45 nM in the Free GSH and 48 nM in the Total GSH assays.

The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low concentration human serum sample.

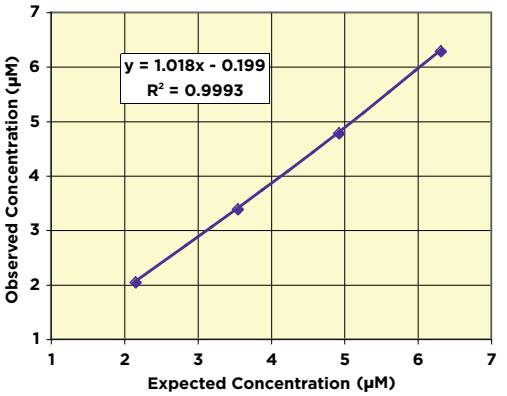
The Limit of Detection was determined as 38 nM in the Free GSH and 42 nM in the Total GSH assays.

Linearity

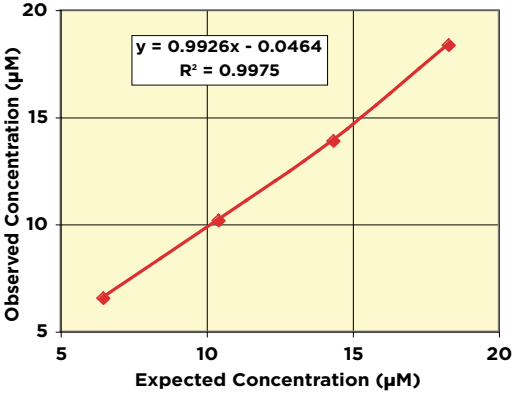
Linearity was determined by taking human RBCs at two different concentrations and mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High RBC Sample	Low RBC Sample	Observed Conc. (µM)		Expected Conc. (µM)		% Recovery	
		Free	Total	Free	Total	Free	Total
100%	0%	7.71	22.23	--	--	--	--
80%	20%	6.28	18.35	6.32	18.29	99.3%	100.3%
60%	40%	4.77	13.89	4.93	14.35	96.7%	96.8%
40%	60%	3.38	10.18	3.55	10.41	95.3%	97.8%
20%	80%	2.04	6.55	2.16	6.47	94.5%	101.2%
0%	100%	0.77	2.53	--	--	--	--
				Mean Recovery		**96.5%**	**99.0%**

Free GSH Linearity



Total GSH Linearity



Intra Assay Precision

Two each of SSA treated human urine and whole blood samples were further diluted in 1% SSA Sample Diluent and run in replicates of 20 in an assay. The mean and precision of the calculated GSH concentrations were:

Sample	GSH Conc. (µM)		%CV	
	Free	Total	Free	Total
1	1.27	2.30	4.0	4.7
2	2.00	3.80	3.1	4.7
3	8.33	9.77	4.6	2.7
4	3.89	4.45	3.0	2.3

Inter Assay Precision

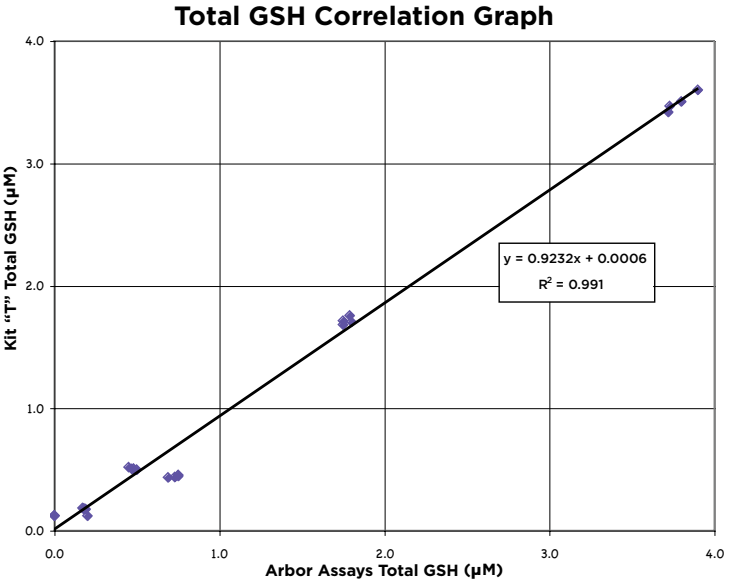
Two each of SSA treated human urine and blood samples were further diluted in 1% SSA Sample Diluent and run in duplicates in twenty assays run over multiple days by two operators. The mean and precision of the calculated GSH concentrations were:

Sample	GSH Conc. (µM)		%CV	
	Free	Total	Free	Total
1	1.30	2.40	8.6	8.3
2	1.83	3.57	14.7	10.0
3	9.38	11.67	6.0	6.0
4	4.89	5.89	7.2	8.0

Kit Correlation Data

We compared a popular colorimetric total glutathione assay kit (kit "T") that uses Ellman's reagent to detect free glutathione in the sample. Initial experiments used random human urine samples that were processed as described in each kit insert. With kit "T", the values obtained for urine after the recommended treatment with 4 volumes of 5% metaphosphoric acid and subsequent 10 fold dilution with assay buffer put all the values well below the lowest standard. However, the urine samples run in the DetectX® kit gave Total GSH values between 0.63 and 4.04 µM.

We also took a Jurkat cell pellet and processed the cells either through the 5% metaphosphoric acid treatment for the kit "T" Ellman's based test or as described on page 9 for the DetectX® kit. Cell samples ranged from 25 to 0.78 x 10^6 cell/mL. Twenty-four samples were run according to manufacturers directions for both kits and the correlation of these samples is shown below.



Many of the cell lysate values for the Ellman's based kit, kit "T", read either below the lowest standard (0.25 µM) or above the highest one (2 µM). This data was calculated via extrapolation from the kinetic method required by kit "T". The lysate values for the DetectX® kit were calculated directly from the endpoint standard curve.

Limited Warranty

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

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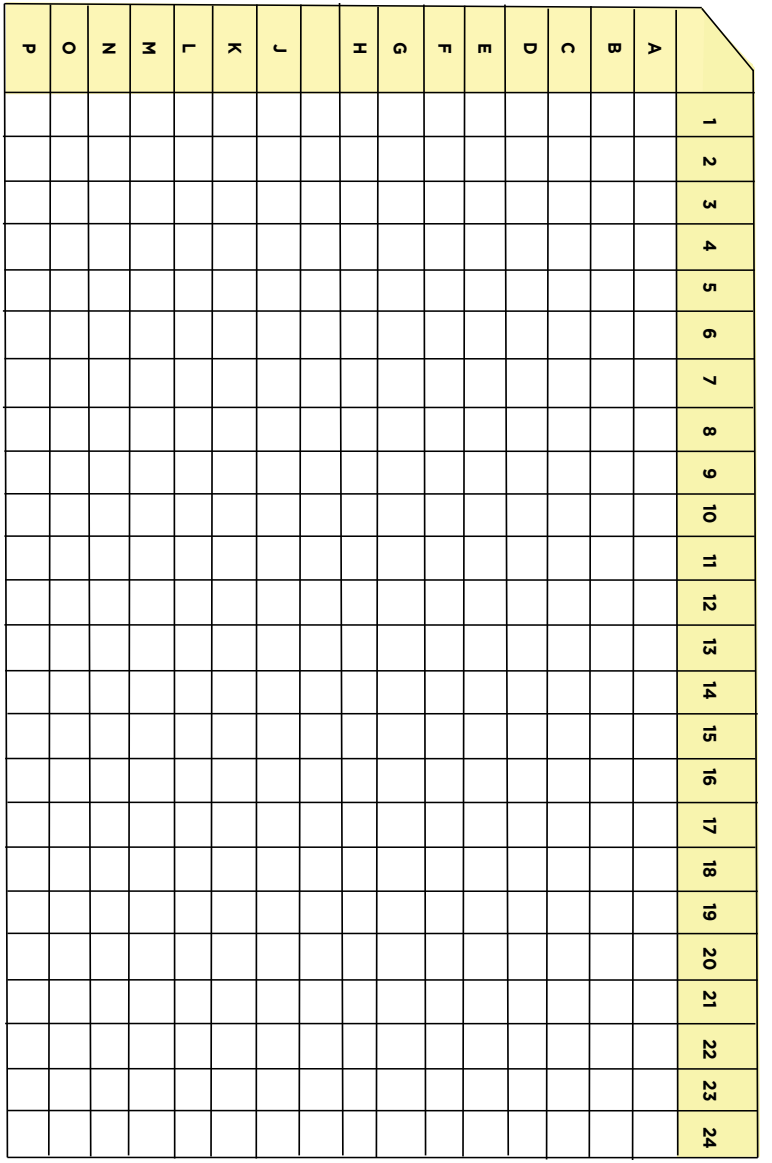

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