



ARBOR ASSAYS™  
Interactive Assay Solutions™



**DetectX<sup>®</sup>**  
**GLUTATHIONE**  
**Colorimetric Cuvette Detection Kit**

2 Plate Kit K006-H1C-L/-H\*

*\* -L/-H refer to Spectrophotometer beam height  
L is for 8.5 mm, H for 15 mm height  
Refer to instrument manual for correct beam height*

**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](http://www.ArborAssays.com)   

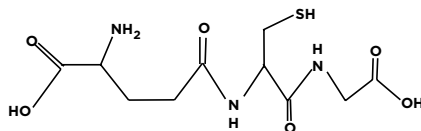
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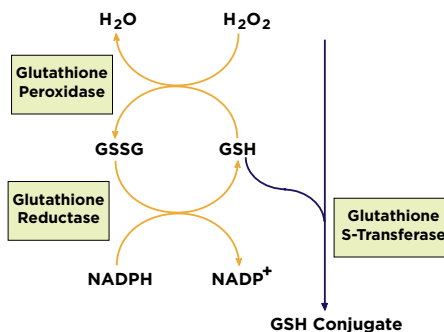


## BACKGROUND

Glutathione (L- $\gamma$ -glutamyl-L-cysteinylglycine; GSH) is the highest concentration non-protein thiol in mammalian cells and is present in concentrations of 0.5 – 10 mM<sup>1</sup>. 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<sup>2</sup>.



The oxidized GSH dimer, GSSG, is formed from GSH and peroxide by the GP reaction (see below). An important role of GSSG in the NFK $\beta$  activating signal cascade is suggested by the facts that the potent NFK $\beta$  inducer, tetradecanoyl phorbol acetate, increases intracellular GSSG levels and GSSG/GSH ratios<sup>3</sup>.



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<sub>4</sub> (LTA<sub>4</sub>). LTA<sub>4</sub> is either hydrated to form LTB<sub>4</sub> or it is conjugated to GSH by a GST, leukotriene C<sub>4</sub> synthase, to form leukotriene C<sub>4</sub>. LTC<sub>4</sub> and its derivative LTD<sub>4</sub> are important molecules in bronchial asthma. Leukotriene C<sub>4</sub> 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. (1988). On the discovery of glutathione. *Trends in Biochemical Sciences*, 13(5), 185-188.
2. Meister, A. (1994). The glutathione-ascorbic acid antioxidant systems in animals. *Journal of Biological Chemistry*, 269, 9397-9400.
3. Dröge, W., et al. (1994). Functions of glutathione and glutathione disulfide in immunology and immunopathology. *The FASEB Journal*, 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. No separation or washing is required. Please read the complete kit insert before performing this assay. 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 plate-based 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. We also sell a plate based Colorimetric Detection kit, K006-H1, that utilizes the same reagents as this kit.

## RELATED PRODUCTS

Kits	Catalog No.
<b>Glutathione Fluorescent Detection Kit</b>	K006-F1/F5
<b>Glutathione S-Transferase Fluorescent Activity Kit</b>	K008-F1
<b>Glutathione Reductase Fluorescent Activity Kit</b>	K009-F1

Reagents	Catalog No.
<b>Glutathione Mouse Monoclonal Antibody, 50 µg</b> Mouse IgG2a, Clone L4H raised to glutathione conjugated to KLH Applications: Western blotting, Immunoassay and Immunoprecipitation	A001-50UG
<b>DyLight® 488 Glutathione Mouse Monoclonal Antibody, 50 µg</b> Purified monoclonal labeled with a stable FITC like fluorescent dye Applications: Flow cytometry and direct immunofluorescence	A001F-50UG



## SUPPLIED COMPONENTS

### Ultra-Micro Cuvettes, 2 by 100 Cuvettes

Cuvettes designed for spectrophotometers with a 8.5 or 15 mm beam height.

8.5 mm Beam Height	Kit K006-H1-L	2 Packages	Catalog Number X096-100EA
15 mm Beam Height	Kit K006-H1-H	2 Packages	Catalog Number X097-100EA

### Oxidized Glutathione Standard

Oxidized Glutathione at 250  $\mu$ M in a special stabilizing solution.

350 $\mu$ L	Catalog Number C020-350UL
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### Detection Reagent Concentrate

Detection substrate in DMSO.

1 mL	Catalog Number X041-1ML
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### Assay Buffer

A phosphate buffer containing chelators and stabilizers.

225 mL	Catalog Number X040-225ML
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### NADPH Concentrate

Reduced  $\beta$ -nicotinamide adenine dinucleotide 2'-phosphate (NADPH) as a stable solution.

1 mL	Catalog Number X043-1ML
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### Glutathione Reductase Conc.

Glutathione Reductase (GR) as a stable solution.

1.4 mL	Catalog Number X130-1.4ML
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## STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

## OTHER MATERIALS REQUIRED

Distilled or deionized water.

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 Catalog Number S2130.

2-Vinylpyridine (2VP) is used to block any free GSH or other thiols present in the treated samples. 2VP is prepared by adding 27  $\mu$ L of 2-vinylpyridine (such as Sigma Catalog Number 132292) to 98  $\mu$ L of ethanol. Use immediately and discard remaining unused solutions.

A UV/Visible spectrophotometer capable of reading optical absorption at 405-412 nm with a beam height of 8.5 mm for use with kit, K006-H1C-L. Or,

A UV/Visible spectrophotometer capable of reading optical absorption at 405-412 nm with a beam height of 15 mm for use with kit, K006-H1C-H.

For information please contact your equipment manufacturer.

Software for converting raw optical density readings and carrying out four parameter logistic curve (4PLC) fitting such as AssayZap™ ([www.biosoft.com/w/assayzap.htm](http://www.biosoft.com/w/assayzap.htm)) from Biosoft.

## 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.



## 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  $-70^{\circ}\text{C}$  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

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  $\mu\text{L}$  of 2VP solution for every 250  $\mu\text{L}$  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  $4^{\circ}\text{C}$ . Centrifuge at 14,000 rpm for 10 minutes at  $4^{\circ}\text{C}$ . Collect the supernatant. If the supernatant contains particulates, re-centrifuge the supernatant for 15 minutes and collect the clarified second supernatant. Samples can be stored in aliquots at  $\leq -70^{\circ}\text{C}$  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  $\geq 1:100$ . For Treated Plasma and Treated Urine a final dilution of  $\geq 1:5$  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  $\mu$ L 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  $\mu$ L 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 250  $\mu$ L RBC's with 1mL of cold 5% SSA. Incubate for 10 minutes at 4°C and 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% and the sample will have been diluted 1:20 at this point. Further dilutions are made in Sample Diluent for a recommended final dilution of  $\geq$  1:40.

## Cell Lysates

Washed cell pellets are resuspended at 1-10x10<sup>6</sup> cells/mL in cold 5% SSA (we used Jurkats at 5x10<sup>6</sup> 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 deproteinized 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  $\geq$  1:20.

**Use all samples within 2 hours of dilution.**





## REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. 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 > 4. 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**

	25 Tubes	50 Tubes	100 Tubes	200 Tubes
<b>Colorimetric Detection Concentrate</b>	140 µL	260 µL	500 µL	1 mL
<b>Assay Buffer</b>	1.26 mL	2.34 mL	4.5 mL	9 mL
<b>Total Colorimetric Reagent Volume</b>	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.

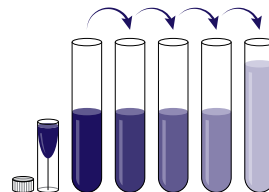
**Reaction Mix Dilution Table**

	25 Tubes	50 Tubes	100 Tubes	200 Tubes
<b>NADPH Concentrate</b>	140 µL	260 µL	500 µL	1 mL
<b>Glutathione Reductase Concentrate</b>	140 µL	260 µL	500 µL	1 mL
<b>Assay Buffer</b>	1.12 mL	2.08 mL	4 mL	8 mL
<b>Total Reaction Mix Volume</b>	1.4 mL	2.6 mL	5 mL	10 mL

## Standard Preparation

**For the measurement of Oxidized Glutathione (GSSG)**, a 50  $\mu\text{L}$  aliquot of the 250  $\mu\text{M}$  Oxidized Glutathione Standard should be treated with 1  $\mu\text{L}$  of 2VP as outlined on page 9. 2VP-treated Standards are prepared by labeling test tubes as #1 through #6. Pipet 475  $\mu\text{L}$  of Sample Diluent into tube #1 and 250  $\mu\text{L}$  into tubes #2 to #6. Carefully add 25  $\mu\text{L}$  of the 2VP-treated Standard to tube #1 and vortex completely. Take 250  $\mu\text{L}$  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  $\mu\text{M}$ . The concentration of Total GSH in tubes 1 through 6 will be 25, 12.5, 6.25, 3.125, 1.56, and 0.781  $\mu\text{M}$  after addition of the Reaction Mixture. 2VP treated Sample Diluent **must** be used as a 0  $\mu\text{M}$  standard.

**To determine Total GSH**, the Standards are prepared by labeling test tubes as #1 through #6. Pipet 475  $\mu\text{L}$  of Sample Diluent into tube #1 and 250  $\mu\text{L}$  into tubes #2 to #6. Carefully add 25  $\mu\text{L}$  of the supplied Standard to tube #1 and vortex completely. Take 250  $\mu\text{L}$  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  $\mu\text{M}$  after addition of the Reaction Mixture. Sample Diluent **must** be used as a 0  $\mu\text{M}$  standard.



**Use all Standards within 2 hours of preparation.**

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
<b>Sample Diluent Volume (<math>\mu\text{L}</math>)</b>	<b>475</b>	250	250	250	250	250
<b>Addition</b>	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
<b>Volume of Addition (<math>\mu\text{L}</math>)</b>	<b>25</b>	250	250	250	250	250
<b>GSSG Conc (<math>\mu\text{M}</math>)</b>	12.5	6.25	3.125	1.56	0.781	0.391
<b>Total GSH Conc (<math>\mu\text{M}</math>)</b>	25	12.5	6.25	3.125	1.56	0.781

## ASSAY PROTOCOL - END POINT

**We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine GSH concentrations.**

**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 cuvette identification sheet on the back page to aid in proper sample and standard identification.
2. Pipet 100  $\mu$ L of either 2VP treated or untreated samples or standards into duplicate cuvettes.
3. Pipet 100  $\mu$ L of either 2VP treated or untreated Sample Diluent into duplicate cuvettes as the Zero standard.
4. Add 50  $\mu$ L of the Colorimetric Detection Reagent to each cuvette using a repeater pipet
5. Add 50  $\mu$ L of the Reaction Mixture to each of the cuvettes using a repeater pipet.
6. Gently tap the sides of the cuvettes 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 cuvettes to ensure adequate mixing of the reagents.
3. Add 50  $\mu$ L of the Reaction Mixture to each of the cuvettes using a repeater and immediately place cuvettes in reader and read optical density at 405 nm every minute for at least 10 minutes. These data will be used to kinetically determine Total or Oxidized Glutathione concentration.

## 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.

Or use the online tool from MyAssays to calculate the data:

[www.myassays.com/arbor-assays-glutathione-colorimetric-detection-kit.assay](http://www.myassays.com/arbor-assays-glutathione-colorimetric-detection-kit.assay)

Glutathione concentrations (see below) are calculated from the data using the curve fitting routine supplied with the plate reader.

Oxidized Glutathione concentrations of the samples are determined from the data obtained from 2VP-treated samples read off a 2VP-treated standard curve. The concentration of Oxidized Glutathione (GSSG) in the samples would be half of the GSH concentration read off the curve.

**Note: 1 GSSG = 2 GSH**

Free glutathione (GSH) concentrations are obtained by subtracting the Oxidized Glutathione (GSSG) levels obtained from the 2VP treated standard and samples from non-treated standards and samples (Total GSH). Concentrations obtained will be in  $\mu\text{M}$  of Glutathione.

**Total GSH = Free GSH + Oxidized GSH (GSSG)**

**Oxidized GSH =  $\frac{\text{(measured 2VP Treated GSH concentration)}}{2}$**

**Free GSH = Total GSH Conc. - Oxidized GSH Conc.**

**All the data presented following on pages 13-17 was generated using our plate based detection kit, K006-H1, which uses identical reagents.**



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ASSAYS

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K006-H1C WEB 200615

EXPECT ASSAY ARTISTRY

## TYPICAL DATA - TOTAL GLUTATHIONE

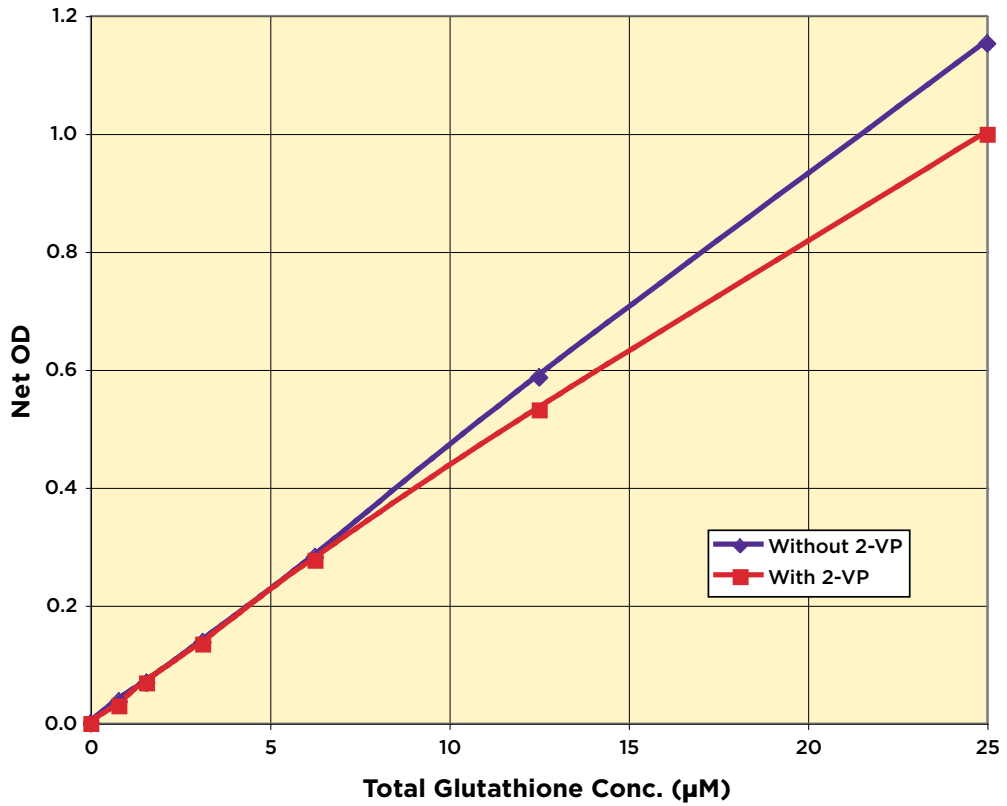
Sample	Mean OD	Net OD	Total Glutathione Conc. (µM)
Zero	0.086	0	0
Standard 1	1.239	1.153	25
Standard 2	0.673	0.587	12.5
Standard 3	0.368	0.282	6.25
Standard 4	0.224	0.138	3.125
Standard 5	0.155	0.069	1.56
Standard 6	0.123	0.037	0.781
Sample 1	0.360	0.273	6.05
Sample 2	0.246	0.160	3.65

## TYPICAL DATA - OXIDIZED GLUTATHIONE

Sample	Mean OD	Net OD	Total Glutathione Conc. (µM)	Oxidized Glutathione Conc. (µM)
Zero	0.087	0	0	0
Standard 1	1.086	0.999	25	12.5
Standard 2	0.619	0.532	12.5	6.25
Standard 3	0.364	0.277	6.25	3.125
Standard 4	0.222	0.135	3.125	1.52
Standard 5	0.156	0.069	1.56	0.781
Standard 6	0.117	0.030	0.781	0.391
Sample 1	0.177	0.089	-	1.02
Sample 2	0.125	0.038	-	0.48

**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 this data.

## VALIDATION DATA

### **Sensitivity and Limit of Detection**

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

**Sensitivity was determined as 0.634  $\mu\text{M}$  of Glutathione. This is equivalent to 31.7  $\text{pM}/\text{well}$ .**

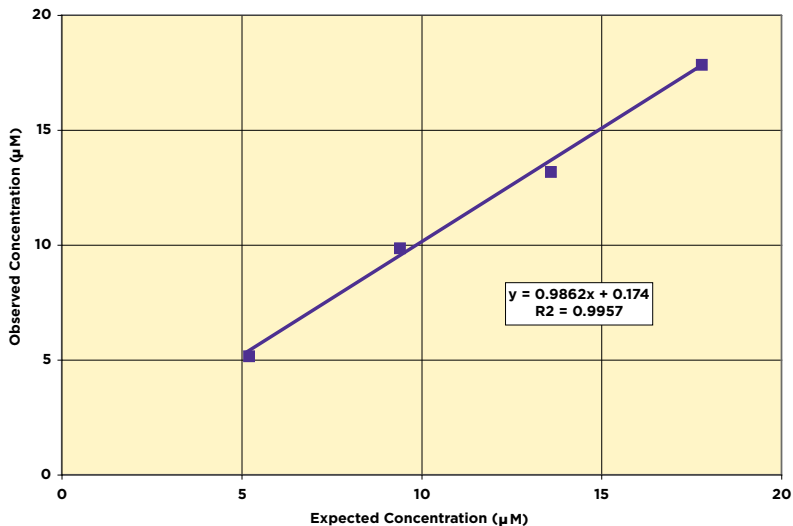
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human urine sample.

**The Limit of Detection was determined as 1.78  $\mu\text{M}$  Glutathione. This is equivalent to 89  $\text{pM}/\text{well}$ .**

## Linearity

Linearity was determined by taking Jurkat cell lysates at  $25 \times 10^6$  cells/mL and one at  $1.28 \times 10^6$  cells/mL, diluted 1:5, and mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Cell #	High Cell #	Observed GSH Conc. ( $\mu\text{M}$ )	Expected GSH Conc. ( $\mu\text{M}$ )	% Recovery
100%	0%	1.01	--	--
80%	20%	5.20	5.16	99.2
60%	40%	9.40	9.86	104.9
40%	60%	13.59	13.18	97.0
20%	80%	17.79	17.84	100.3
0%	100%	21.98	--	--
<b>Mean Recovery</b>				<b>100.3%</b>





### Intra Assay Precision

Two whole blood samples and one human urine sample were SSA treated, diluted in 1% SSA Standard Diluent and run in replicates of 20 in an assay. The mean and precision of the calculated Total Glutathione concentrations were:

Sample	Total Glutathione Conc. (µM)	%CV
1	9.36	2.1
2	5.55	3.1
3	3.30	5.0

### Inter Assay Precision

Two whole blood samples and one human urine sample were SSA treated , diluted in 1% SSA Standard Diluent and run in duplicates in twenty assays run over multiple days by four operators. The mean and precision of the calculated Total Glutathione concentrations were:

Sample	Total Glutathione Conc. (µM)	%CV
1	9.43	7.5
2	5.27	8.4
3	3.08	13.3

## REPORTED NORMAL RANGES

Reference	Sample	Total GSH	Free GSH	GSSG
Clinical Chemistry (2002) 48/5:742-53	Whole blood	914 – 996 ± 101 µM	756-945 ± 95 µM	5.66-6.6 ± 1.55 µM
Internet Journal of Alternative Medicine (2005) 2/1	EDTA Erythrocytes		95.2 ± 11.5 µM (n=45)	
Diabetes (1999) 48:1850-55	Erythrocytes		5.7 ± 0.3 µM/g Hgb	
Clinical Chemistry (2002) 48/5:742-53	Erythrocytes			3.27 ± 1.15 µM
J Biol. Chem. (1929) 83/2:-361-65	EDTA Plasma	7.3 ± 1.8 µM	3.4 ± 0.9 µM	
Clinical Chemistry (1987) 33/9:1675-76	Plasma	3.35 ± 1.07 µM		0.35 ± 0.14 µM
J Biol. Chem. (2007) 282/19:14337-4	Jurkat Lysate		40 ng/ mg total Protein	
FASEB J. (2000) 14:1352-61	Jurkat Lysate		15 ng/mg total Protein	

## 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.

## CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

### Arbor Assays

1514 Eisenhower Place  
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: [www.ArborAssays.com](http://www.ArborAssays.com)

### E Mail Addresses:

[Info@ArborAssays.com](mailto:Info@ArborAssays.com)

[Orders@ArborAssays.com](mailto:Orders@ArborAssays.com)

[Technical@ArborAssays.com](mailto:Technical@ArborAssays.com)

[Contracts@ArborAssays.com](mailto:Contracts@ArborAssays.com)



## OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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