



ARBOR
ASSAYS™

DetectX® Glutathione
Colorimetric Detection Kit

4 Plate Kit – Catalog No. K006-H1

Species Independent

Sample Types Tested:

*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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SUPPLIED COMPONENTS & STORAGE

| K006-H1 | | Description |
|-----------------------------------|-------------|-------------|
| Clear Half Area 96-well Plate | Quantity | 4 |
| | Catalog No. | X018-4EA |
| Oxidized Glutathione Standard | Volume | 350 μ L |
| | Catalog No. | C020-350UL |
| Detection Reagent Concentrate | Volume | 1 mL |
| | Catalog No. | X041-1ML |
| Assay Buffer | Volume | 225 mL |
| | Catalog No. | X040-225ML |
| NADPH Concentrate | Volume | 1 mL |
| | Catalog No. | X043-1ML |
| Glutathione Reductase Concentrate | Volume | 1 mL |
| | Catalog No. | X130-1ML |

Oxidized Glutathione at 250 μ M in stabilizing solution

Detection substrate in DMSO

A phosphate buffer containing chelators and stabilizers

Reduced β -nicotinamide adenine dinucleotide 2'-phosphate (NADPH) as a stable solution.
Must be stored at -20°C.

Glutathione Reductase (GR) as a stable solution

The unopened kit must be stored at -20°C.

Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the NADPH Concentrate which must be stored at -20°C.**

OTHER MATERIALS REQUIRED

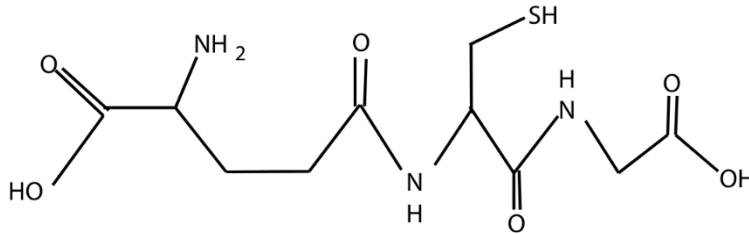
- Distilled or deionized water
- Adjustable pipettes with disposable tips. Repeater pipettes or multichannel pipettes with corresponding tips are also recommended.
- Glass or high-quality polypropylene test tubes for Standard and Sample preparation
- A plate reader capable of measuring absorbance at 405 nm
- Aqueous 5-sulfo-salicylic acid dihydrate (SSA, Sigma-Aldrich Cat. S2130 or similar) solution at 5% weight/volume (1g of SSA per 20 mL of water) for treating samples to remove protein.
- 2-Vinylpyridine (2VP, such as Sigma Catalog Number 132292) if measuring Oxidized Glutathione
- **Tissue Samples and Cell Lysates:** 1X phosphate buffered saline (PBS).
- **Erythrocytes/Red Blood Cells:** Heparin or EDTA for blood collection. Isotonic saline (0.9%) for washing.
- pH strips or pH meter
- Fume Hood

PRECAUTIONS

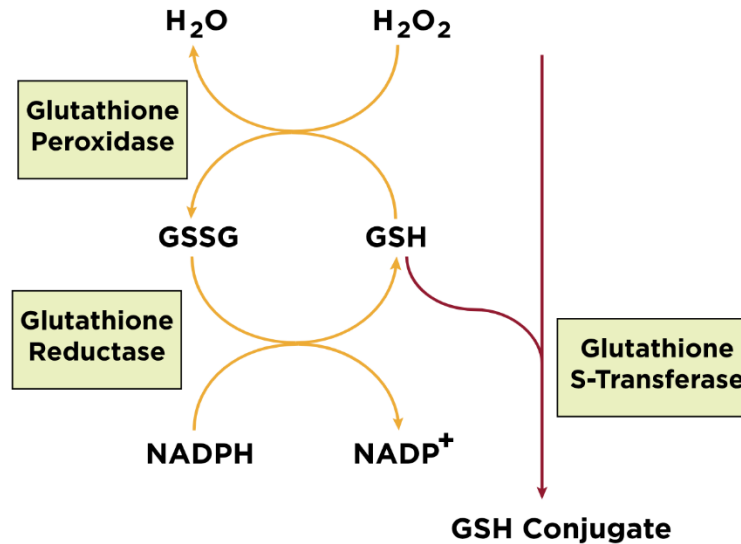
- Read this insert completely prior to using the product.
- This kit may not perform as described if any reagent or procedure is replaced or modified. Do not interchange reagents from different kit lots.
- Take appropriate safety precautions, such as: avoid breathing fumes, wear personal protective equipment (gloves, clothing, eye and face protection), and familiarize yourself with SDS documents.
 - https://www.arborassays.com/documentation/msds/K006-H1_MSDS.pdf
- 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 (DMSO) is a powerful aprotic organic solvent shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent.

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.



The oxidized GSH dimer, GSSG, is formed from GSH and peroxide by the Glutathione Peroxidase reaction (see below). An important role of GSSG in the NF- κ B activating signal cascade is suggested by the facts that the potent NF- κ B inducer, tetradecanoyl phorbol acetate, increases intracellular GSSG levels and GSSG/GSH ratios³.



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 several diseases. In asthma arachidonic acid is converted to unstable leukotriene A₄ (LTA₄). LTA₄ is either hydrated to form LTB₄ or it is conjugated to GSH by a GST, leukotriene C₄ synthase, to form leukotriene C₄. LTC₄ and its derivative LTD₄ are important molecules in bronchial asthma. Leukotriene C₄ 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.

ASSAY PRINCIPLE

The DetectX® Glutathione kit is designed to quantitatively measure total 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 measured Total GSH and the Oxidized Glutathione from the 2-Vinylpyridine treatment. The concentration of GSH can be determined either as an endpoint read of the color developed at 405 nm or kinetically by measuring the rate of color development at 405 nm.

GSH is identical across species. This assay has been validated for human whole blood, serum, EDTA and heparin plasma, urine, and isolated erythrocytes. Most cell lysates and tissue homogenates are also compatible. All samples should be deproteinized with 5% SSA, which removes protein thiols present in the samples and slows oxidation of free GSH.

REAGENT PREPARATION

Except for the reagents listed below, all kit components are ready for use.

| Reagent | Preparation | Stability |
|--------------------------|--|---|
| Sample Diluent | Mix 1 volume 5% SSA with 4 volumes Assay Buffer. Vortex thoroughly. Note: The pH of the Sample Diluent must be > 4. | Sample Diluent can be stored at 4°C for one month. |
| Detection Reagent | Mix 1 volume Detection Reagent Concentrate with 9 volumes Assay Buffer. For one plate, add 260 µL Detection Reagent Concentrate to 2.34 mL Assay Buffer for a total volume of 2.6 mL. Scale accordingly. | Store unused Detection Reagent at 4°C in an amber vial for no more than 2 days. |
| Reaction Mixture | Mix 1 volume NADPH, 1 volume Glutathione Reductase Concentrate, and 8 volumes Assay Buffer. For one plate, add 260 µL NADPH and 260 µL Glutathione Reductase Concentrate to 2.08 mL Assay Buffer for a total volume of 2.6 mL. Scale accordingly. | Store unused Reaction Mixture at 4°C in an amber vial for no more than 2 days. |
| 2VP | Add 27 µL of 2-vinylpyridine to 98 µL 100% ethanol. Scale volumes as needed. | Use immediately and discard any remaining unused solution. |

SAMPLE PREPARATION

For samples containing particulates, centrifuge prior to use. Upon collection, all samples should be frozen rapidly and stored at -80°C until testing.

All SSA treated centrifuged supernatants must have their SSA concentration brought down to 1% SSA by dilution with Assay Buffer, see detailed instructions below.

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

| Sample Type | Procedure |
|---|--|
| Whole Blood, Serum, EDTA or Heparin Plasma, or Urine | <ol style="list-style-type: none"> 1. Mix sample with equal volumes cold 5% SSA. Incubate 10 minutes at 4°C. 2. Centrifuge at 20,800 RCF for 10 minutes at 4°C. Collect the supernatant. <ul style="list-style-type: none"> • If the supernatant contains particulates, centrifuge the supernatant again for 15 minutes and collect the clarified second supernatant. 3. Samples can be stored in aliquots at -80°C or analyzed immediately (SSA concentration = 2.5%). 4. Further dilute supernatant by adding 1 volume of sample with 1.5 volumes of Assay Buffer (SSA concentration = 1%, sample dilution = 1:5). 5. Whole Blood: Further dilute at least 1:20 in Sample Diluent to recommended final dilution of ≥ 1:100. Plasma, Serum, and Urine: Further dilutions in Sample Diluent may be necessary to fall within the standard curve range. |

1. Wash fresh tissue samples with ice cold PBS to remove blood. Blot 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:

2. Homogenize at 10 mg/250 μ L in ice cold 100mM phosphate buffer, pH 7.
3. Centrifuge at 20,800 RCF for 10 minutes at 4°C and remove an aliquot of the supernatant for protein determination.
4. Thoroughly mix a second aliquot of the supernatant with an equal volume of cold 5% SSA.
5. Incubate for 10 minutes at 4°C.
6. Centrifuge at 20,800 RCF for 10 minutes at 4°C to remove precipitated protein. Collect the supernatant.
7. Further dilute supernatant 1:2.5 with Assay Buffer by mixing one part sample with 1.5 parts of Assay Buffer (SSA concentration = 1%).

Tissue Samples

For Samples Not Requiring a Protein Determination:

2. Homogenize at 10 mg/250 μ L in ice cold 5% SSA.
3. Incubate at 10 minutes at 4°C.
4. Centrifuge at 20,800 RCF for 10 minutes at 4°C.
5. Collect the supernatant.
6. Further dilute supernatant 1:5 with Assay Buffer by mixing one volume supernatant with 4 volumes Assay Buffer (SSA concentration = 1%).

Additional sample dilutions must be done in **Sample Diluent**, and the appropriate dilution will depend on the tissue type and the amount of tissue being assayed.

Erythrocytes/Red Blood Cells (RBCs)

1. Collect blood with heparin or EDTA.
2. Centrifuge the sample, remove and discard the plasma and white cell layer.
3. Wash the RBC's 2 times by suspending in 3 volumes of isotonic saline (0.9%), centrifuging at 600 RCF for 10 minutes and discarding the supernatant.
4. After the 2 washes, mix 250 μ L RBCs with 1 mL of cold 5% SSA.
5. Incubate for 10 minutes at 4°C and centrifuge at 20,800 RCF for 10 minutes at 4°C. Collect the supernatant (SSA concentration = 4%).
6. Dilute the supernatant 1:4 with Assay Buffer by mixing one volume supernatant with 3 volumes Assay Buffer (SSA concentration = 1%; sample dilution = 1:20).
7. Further dilute in **Sample Diluent** for a recommended final dilution of $\geq 1:40$.

Cell Lysates

1. Wash cell pellets and resuspend at 1×10^6 to 10×10^6 cells/mL in cold 5% SSA. **Note:** Kit was validated with Jurkat cells at 5×10^6 cells/mL.
2. Lyse and deproteinize cells by vigorous vortexing, freeze/thaw cycling, or other suitable disruption method.
3. Incubate cells at 4°C for 10 minutes.
4. Centrifuge for 10 minutes at 20,800 RCF and 4°C.
5. Further dilute deproteinized supernatant 1:5 with Assay Buffer by mixing one part with 4 parts of Assay Buffer. The SSA concentration will be 1%.

Additional sample dilutions must be done in **Sample Diluent** and the appropriate dilution will depend on the cell type and number of cells being assayed. The recommended final dilution is $\geq 1:20$.

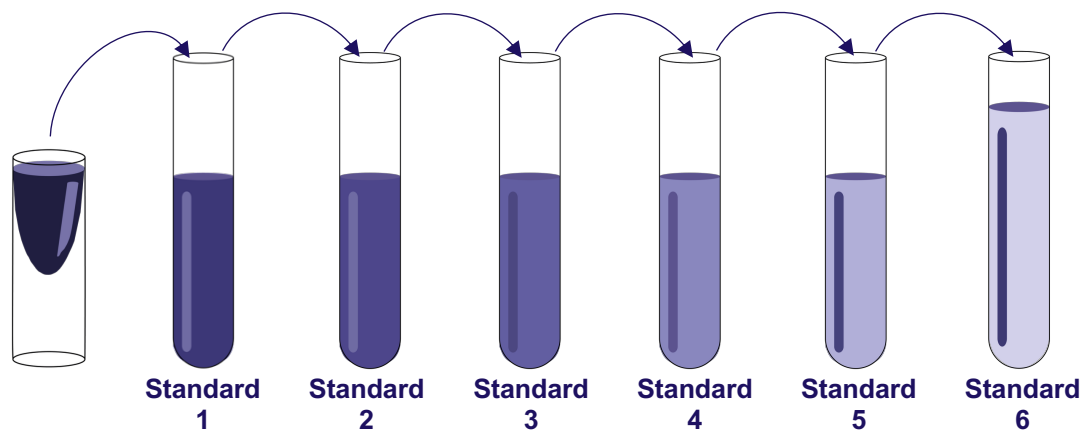


Use all samples within 2 hours of dilution.

TOTAL GLUTATHIONE (GSH) ASSAY

TOTAL GSH STANDARD PREPARATION

1. Label tubes Standard 1 through Standard 6.
2. Add 475 μL Sample Diluent to Standard 1 tube.
3. Add 250 μL Sample Diluent to Standard 2 – 6 tubes.
4. Briefly vortex the Oxidized Glutathione Standard stock and add 25 μL to Standard 1 tube. Vortex thoroughly.
5. Transfer 250 μL of the mixed solution from Standard 1 into Standard 2 tube. Vortex thoroughly.
6. Transfer 250 μL of the mixed solution from Standard 2 into Standard 3 tube. Vortex thoroughly.
7. Continue serially diluting into the remaining tubes. This process and the final concentrations are summarized in the table below.
8. Sample Diluent must be used as a 0 μM standard.



| 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 |
| Total GSH Concentration (μM) | 25.0 | 12.5 | 6.25 | 3.13 | 1.56 | 0.78 |

⚠ Use all Standards within 2 hours of dilution.

TOTAL GSH ASSAY PROTOCOL

Before You Begin:

- **Room Temperature for this assay is defined as 22°C – 24°C.**
- **Ensure all reagents have been warmed to room temperature.**
- **Dilute samples as described in Sample Preparation.**
- **Run all standards and samples in duplicate.**
- Use the blank plate template on the back page of this booklet to design your plate layout and aid in proper sample and standard identification.
- Set plate parameters on the plate reader for a 96-well Corning Costar 3695 plate. See ArborAssays.com for plate dimension data.

END POINT PROTOCOL

1. Add 50 µL Samples or Standards into duplicate wells.
2. Add 50 µL Sample Diluent into duplicate Zero Standard wells.
3. Add 25 µL Colorimetric Detection Reagent to each well.
4. Add 25 µL Reaction Mixture to each well.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 20 minutes.
7. Read the optical density at 405 nm. These data will be used to determine the Total Glutathione concentration (see page 13).

KINETIC PROTOCOL

1. Add 50 µL Samples or Standards into duplicate wells.
2. Add 50 µL Sample Diluent into duplicate wells as the Zero standard.
3. Add 25 µL Colorimetric Detection Reagent to each well.
4. Add 25 µL Reaction Mixture to each well.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Immediately place plate in reader.
7. Read the optical density at 405 nm every minute for at least 10 minutes. These data will be used to kinetically determine the Total Glutathione concentration (see page 13).

OXIDIZED GLUTATHIONE (GSSG) ASSAY

2-Vinylpyridine Treatment (2VP)

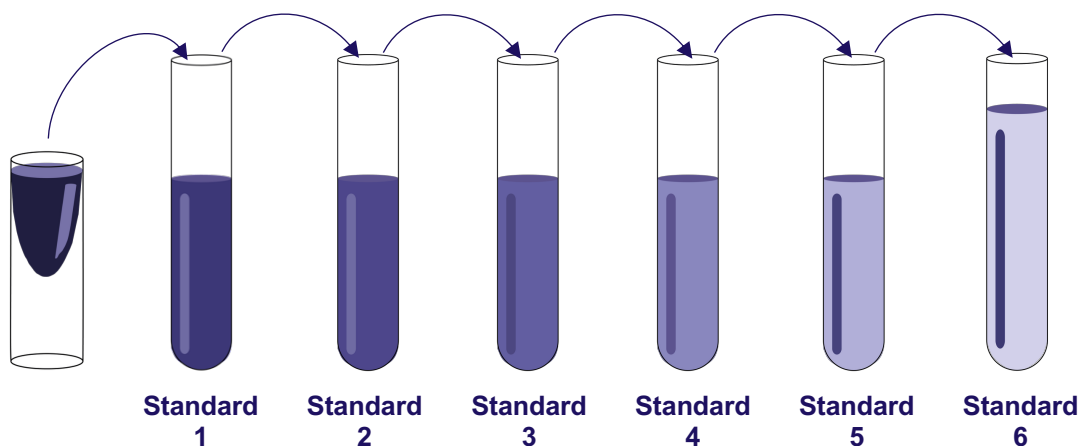
To measure Oxidized Glutathione, free GSH must be blocked by alkylation.

1. Treat Standards, Samples, and Sample Diluent with 2VP
 - **Standards:** Add 1 μL 2VP to 50 μL Oxidized Glutathione Standard Stock.
 - **Samples:** Add 5 μL 2VP to 250 μL of each Sample (2.5% SSA).
 - **Sample Diluent:** Calculate the amount of Sample Diluent required for all Samples and Standards to be run. Add 1 μL 2VP to every 50 μL Sample Diluent. Scale accordingly.
2. Briefly vortex each to mix, then incubate at room temperature for 1 hour.

⚠ Samples treated with 2VP must be read off a standard curve generated with 2VP treated Standards.

GSSG STANDARD PREPARATION

1. Label tubes Standard 1 through Standard 6.
2. Add 475 μL **2VP-treated** Sample Diluent to Standard 1 tube.
3. Add 250 μL **2VP-treated** Sample Diluent to Standard 2 – 6 tubes.
4. Add 25 μL **2VP-treated** Oxidized Glutathione Standard stock solution to Standard 1 tube. Vortex thoroughly.
5. Transfer 250 μL of Standard 1 into Standard 2 tube. Vortex thoroughly.
6. Transfer 250 μL of Standard 2 into Standard 3 tube. Vortex thoroughly.
7. Continue serially diluting into the remaining tubes. This process and the final concentrations are summarized in the table below.
8. **2VP-treated** Sample Diluent must be used as a 0 μM standard.



| | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 |
|---|------------|------------|------------|------------|------------|------------|
| 2VP Treated Sample Diluent Volume (μL) | 475 | 250 | 250 | 250 | 250 | 250 |
| Addition | Stock | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 |
| Volume of Addition (μL) | 25 | 250 | 250 | 250 | 250 | 250 |
| GSSG Concentration (μM) | 12.5 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 |

⚠ Use all Standards within 2 hours of dilution.

Oxidized Glutathione (GSSG) ASSAY PROTOCOL

Before You Begin:

- **Room Temperature** for this assay is defined as 22°C – 24°C.
- **Ensure all reagents have been warmed to room temperature.**
- **Dilute samples as described in Sample Preparation.**
- **Treat Standards, Samples, and Sample Diluent with 2VP prior to running assay.**
- **Run all Standards and Samples in duplicate.**
- Use the blank plate template on the back page of this booklet to design your plate layout and aid in proper sample and standard identification.
- Set plate parameters on the plate reader for a 96-well Corning Costar 3695 plate. See [ArborAssays.com](https://www.arborassays.com) for plate dimension data.

END POINT PROTOCOL

1. Add 50 µL **2VP-treated** Samples or Standards into duplicate wells.
2. Add 50 µL **2VP-treated** Sample Diluent into duplicate Zero Standard wells.
3. Add 25 µL Colorimetric Detection Reagent to each well.
4. Add 25 µL Reaction Mixture to each well.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 20 minutes.
7. Read the optical density at 405 nm. These data will be used to determine the GSSG concentration.

KINETIC PROTOCOL

1. Add 50 µL **2VP-treated** Samples or Standards into duplicate wells.
2. Add 50 µL **2VP-treated** Sample Diluent into duplicate Zero Standard wells.
3. Add 25 µL Colorimetric Detection Reagent to each well.
4. Add 25 µL Reaction Mixture to each well.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Immediately place plate in reader.
7. Read the optical density at 405 nm every minute for at least 10 minutes. These data will be used to kinetically determine the GSSG concentration.

CALCULATION OF RESULTS

Follow the instructions below, or use this online tool: <https://myassays.com/assay.aspx?id=936>

1. Use four-parameter logistic curve (4PL) software to calculate the Glutathione or Oxidized Glutathione concentration for each sample. Gather all raw data OD readings from each Sample and Standard, including the Zero Standard.
2. Average the duplicate OD readings for each Sample, Standard, and Zero Standard (Mean OD).

EXAMPLE:

| Sample | Replicate 1 OD | Replicate 2 OD | Mean OD |
|---------------------|----------------|----------------|---------|
| Zero Standard | 0.076 | 0.096 | 0.086 |
| Standard 1 | 1.235 | 1.243 | 1.239 |
| Sample 1 | 0.355 | 0.365 | 0.360 |
| 2VP – Zero Standard | 0.077 | 0.097 | 0.087 |
| 2VP – Standard 1 | 1.083 | 1.089 | 1.086 |
| 2VP – Sample 1 | 0.173 | 0.181 | 0.177 |

3. Subtract the Zero Standard Mean OD from the Mean OD for each Sample and Standard (Net OD).

EXAMPLE:

| Sample | Mean OD | Zero Standard Mean OD | Net OD |
|------------------|---------|-----------------------|--------|
| Standard 1 | 1.239 | 0.086 | 1.153 |
| Sample 1 | 0.360 | 0.086 | 0.274 |
| 2VP – Standard 1 | 1.086 | 0.087 | 0.999 |
| 2VP – Sample 1 | 0.177 | 0.087 | 0.090 |

4. Plot the standard curve with Net OD for the Standards on the y-axis and Glutathione concentration (μM) on the x-axis. Perform a 4PL fit.

Use the sample Net OD readings and the 4PL fit to calculate the Glutathione concentration in diluted samples. If diluted sample Glutathione concentration is outside of the range of the standards, the sample should be prepared again at a more appropriate dilution. Note that 1 GSSG = 2 GSH, therefore multiply the 2VP treated concentration by 2 to enable the subsequent subtraction.

EXAMPLE:

| Sample | Net OD | Oxidized Glutathione Concentration (μM) | Glutathione Concentration (μM) |
|----------------|--------|--|---|
| Sample 1 | 0.274 | - | 6.05 (Total GSH) |
| 2VP – Sample 1 | 0.090 | 1.02 (GSSG) | 2.04 (Oxidized GSH) |

5. To obtain the Free GSH concentration of the sample, use the formula below:

$$\text{Free GSH} = [\text{Total GSH}] - [\text{Oxidized GSH}]$$

EXAMPLE:

| Sample | Total GSH Concentration (μM) | Oxidized GSH (μM) | Free GSH Concentration (μM) |
|----------|---|--------------------------------|--|
| Sample 1 | 6.05 | 2.04 | 4.01 |

6. If the original sample was diluted, multiply the sample Glutathione concentration by the sample dilution factor to determine the Glutathione concentration in the original sample.

EXAMPLE:

| Sample | Free GSH Concentration (μM) | Sample Dilution Factor | Original Free GSH Concentration (μM) |
|----------|--|------------------------|---|
| Sample 1 | 4.01 | 20 | 80.2 |

TYPICAL DATA

⚠ Always run your own standard curve. This data should NOT be used to interpret results.

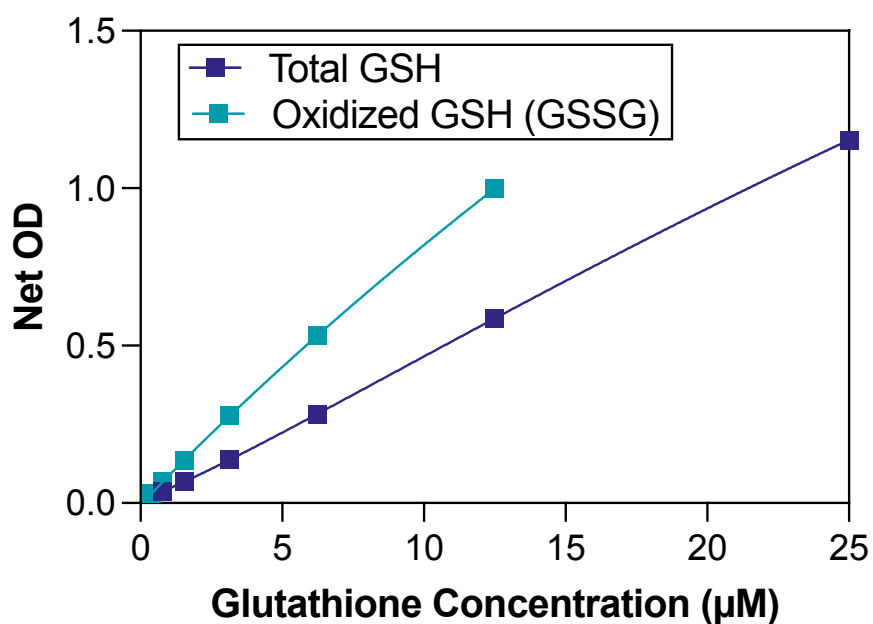
TOTAL GSH

| Sample | Mean OD | Net OD | Total Glutathione Concentration (μM) |
|------------|---------|--------|---|
| Standard 1 | 1.239 | 1.153 | 25.0 |
| Standard 2 | 0.673 | 0.587 | 12.5 |
| Standard 3 | 0.368 | 0.282 | 6.25 |
| Standard 4 | 0.224 | 0.138 | 3.13 |
| Standard 5 | 0.155 | 0.069 | 1.56 |
| Standard 6 | 0.123 | 0.037 | 0.78 |
| Zero | 0.086 | - | - |
| Sample 1 | 0.360 | 0.274 | 6.05 |
| Sample 2 | 0.246 | 0.160 | 3.65 |

OXIDIZED GSH (GSSG)

| Sample | Mean OD | Net OD | Oxidized Glutathione Concentration (μM) |
|------------|---------|--------|--|
| Standard 1 | 1.086 | 0.999 | 12.5 |
| Standard 2 | 0.619 | 0.532 | 6.25 |
| Standard 3 | 0.364 | 0.277 | 3.13 |
| Standard 4 | 0.222 | 0.135 | 1.56 |
| Standard 5 | 0.156 | 0.069 | 0.78 |
| Standard 6 | 0.117 | 0.030 | 0.39 |
| Zero | 0.087 | - | - |
| Sample 1 | 0.177 | 0.090 | 1.02 |
| Sample 2 | 0.125 | 0.038 | 0.48 |

Typical Standard Curve



VALIDATION DATA

Sensitivity and Limit of Detection

Total GSH Sensitivity was calculated by comparing the ODs for twenty wells run for each of the Zero Standard and Standard #6. The detection limit was determined at two standard deviations from the Zero Standard along the standard curve.

Sensitivity was determined as 0.63 μM of Glutathione (31.7 pM/well).

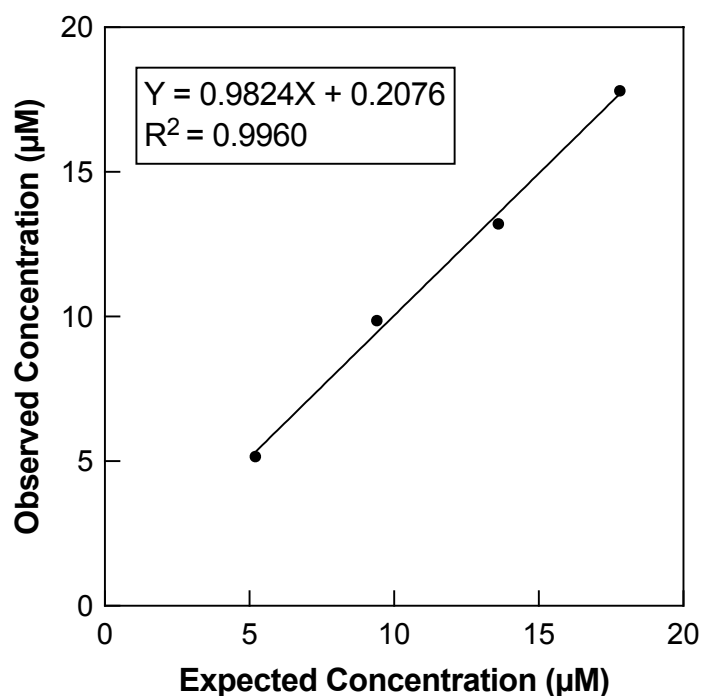
Total GSH Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the Zero Standard and a low concentration human urine sample.

The Limit of Detection was determined as 1.78 μM Glutathione (89 pM/well).

Linearity

Total GSH Linearity was determined in Jurkat cell lysates. One sample had a GSH concentration of 21.98 μM ; a second had a GSH concentration of 1.01 μM . The two samples were mixed in the ratios given below and the measured concentrations were compared to the expected values for each given ratio.

| Low Sample | High Sample | Observed GSH Concentration (μM) | Expected GSH Concentration (μM) | % Recovery |
|---------------|-------------|--|--|------------|
| 80% | 20% | 5.20 | 5.16 | 99.2 |
| 60% | 40% | 9.40 | 9.86 | 104.9 |
| 40% | 60% | 13.6 | 13.2 | 97.0 |
| 20% | 80% | 17.8 | 17.8 | 100.3 |
| Mean Recovery | | | | 100.3% |



Intra Assay and Inter Assay Precision

For intra assay precision, two whole blood samples and one human urine sample were SSA treated, diluted in Sample Diluent and run in replicates of 20 in one assay. For inter assay precision, two whole blood samples and one human urine sample were SSA treated, diluted in Sample Diluent and run in duplicates in twenty assays run over multiple days by four operators. The %CV represents the variation in concentration (not optical density) as determined using a standard curve.

| Sample | Intra Assay Precision | | Inter Assay Precision | |
|--------|------------------------------|------|------------------------------|------|
| | Total GSH Concentration (μM) | % CV | Total GSH Concentration (μM) | % CV |
| 1 | 9.36 | 2.1 | 9.43 | 7.5 |
| 2 | 5.55 | 3.1 | 5.27 | 8.4 |
| 3 | 3.30 | 5.0 | 3.08 | 13.3 |

TROUBLESHOOTING

| Issue | Possible Cause & Solution |
|------------------|--|
| Reagent Shortage | <ul style="list-style-type: none">• Check under the cap for additional reagent. Pulse spin reagent containers to collect contents prior to opening when possible.• When using a multichannel pipette, return unused reagent to container for later use. |
| Erratic Values | <ul style="list-style-type: none">• Prerinse pipet tips with desired reagent prior to aspirating the required volume.• Deliver volume with care to prevent splashing into adjacent wells. |
| High Background | <ul style="list-style-type: none">• Reagent contamination during assay setup. |
| Low/No Signal | <ul style="list-style-type: none">• Verify the plate reader wavelength is 405 nm.• Verify the reaction mixture was prepared in Assay Buffer, not Sample Diluent. |

CITATIONS

1. Meister, A. (1988). On the discovery of glutathione. Trends in Biochemical Science, 13(5), 185-188.
2. Meister, A. (1994). The glutathione-ascorbic acid antioxidant systems in animals. Journal of Biological Chemistry, 269(13), 9397-9400.
3. Dröge W, et al. (1994). Functions of glutathione and glutathione disulfide in immunology and immunopathology. The FASEB Journal, 8, 1131-1138.

RELATED PRODUCTS

| Kits/Reagents | Catalog No. |
|---|-------------|
| Glutathione Fluorescent Detection Kit | K006-F1/F5 |
| Glutathione Reductase Fluorescent Activity Kit | K009-F1 |
| Glutathione Mouse Monoclonal Antibody, 50 µg | A001-50UG |
| DyLight® 488 Glutathione Mouse Monoclonal Antibody, 50 µg | A001F-50UG |

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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CONTACT INFORMATION

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

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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 assay kits and reagents for wildlife conservation research.

PLATE LAYOUT

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |