# Glutathione Colorimetric Detection Kit

**Catalog Number** EIAGSHC (384 tests)

**Rev** 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Product description

The Gluthathione Colorimetric Detection Kit is designed to measure glutathione (GSH) and oxidized glutathione (GSSG) content in a variety of samples. The kit uses a colorimetric substrate that reacts with the free thiol group on GSH to produce a highly colored product.

The assay measures the glutathione content in whole blood, serum, plasma, erythrocytes, urine, cell lysates, and tissue samples. The assay was characterized with human samples, but can be used to test samples from other species. The assay can be run as an end point assay, or as a kinetic activity assay.

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is the highest concentration non-protein thiol in mammalian cells, and is present at concentrations of 0.5–10 mM. GSH plays a key role in many biological processes, including proteins and DNA synthesis, amino acid transport, and oxidative stress protection.

## Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

| Components | Quantity |
|---|---|
| Oxidized Glutathione Standard; 250 µM oxidized glutathione in a special stabilizing solution | 350 µL |
| Clear 96-well Half Area Plate | 4 plates |
| Detection Reagent Concentrate; in DMSO | 1 mL |
| Assay Buffer; phosphate buffer with chelators and stabilizers | 225 mL |
| NADPH Concentrate: reduced β-nicotinamide adenine dinucleotide 2'-phosphate | 1 mL |
| Glutathione Reductase Concentrate | 1 mL |

## Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 405 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Aqueous 5-sulfo-salicylic acid dihydrate (Sigma-Aldrich S2130)
- 2-vinylpyridine (Sigma-Aldrich 132292)
- Ethanol

## Procedural guidelines

- To determine **Free GSH content**, assays must be performed for both the **total GSH** content and **GSSG** content.
- **GSSG** concentrations are determined from the data obtained from 2VP treated samples read from a 2VP treated standard curve.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

## Prepare 5% SSA (w/v)

Add 1 g of aqueous 5-sulfo-salicylic acid dehydrate to 20 mL of water.

## Prepare Sample Diluent

1. Dilute 5% SSA 1:5 with Assay Buffer (e.g., add 5 mL 5% SSA to 20 mL Assay Buffer) and vortex thoroughly.
2. Adjust pH of Sample Diluent to >6.
3. Store the Sample Diluent at 2°C to 8°C for 1 month.

## (*Optional*) Prepare 2-vinylpyridine (2VP) solution

2-vinylpyridine (2VP) is used to block free GSH or other thiols present in samples to determine oxidized glutathione content.

**Important**: 2-vinylpyridine is toxic and can cause burns. Prepare solution in a fume hood.

1. Add 27 µL of 2-vinylpyridine to 98 µL of ethanol.
2. Use immediately and discard remaining unused solutions by mixing with copious amounts of water.

## Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- Deproteinize all samples with 5% SSA. Dilute treated samples with Assay Buffer to 1% SSA.

## Prepare cell lysate samples

The following procedure is used to prepare cell lysate samples. For sample preparation and dilution procedures for other sample types (whole blood, serum, plasma (EDTA and heparin), erythrocytes (RBCs), tissue, or urine), see the product page at **thermofisher.com**

Because conditions may vary, these procedures may require optimization based on sample type. After preparation, store samples on ice until assaying or freeze in aliquots for later use.

1. Wash cell pellets in ice cold PBS and resuspend in ice cold 5% SSA at 1–40 × $10^6$ cells/mL.

   **Note**: Lysed cells in frozen samples can result in substantial amounts of GSH and GSSG in the PBS wash.
2. Lyse cells by vigorous vortexing, freeze-thaw cycling or other suitable disruption method.
3. Incubate for 10 minutes at 4°C.
4. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant for analysis.
5. Proceed to "Dilute samples" if measuring **total GSH content**.
6. (*Optional*) Treat samples with 2VP if measuring **GSSG content**.
   a. Add 5 µL of 2VP solution for every 250 µL of sample, and incubate for 1 hour at room temperature.
   b. Proceed to "Dilute samples".

## Dilute cell lysate samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application. **Use all samples within 2 hours of dilution.**

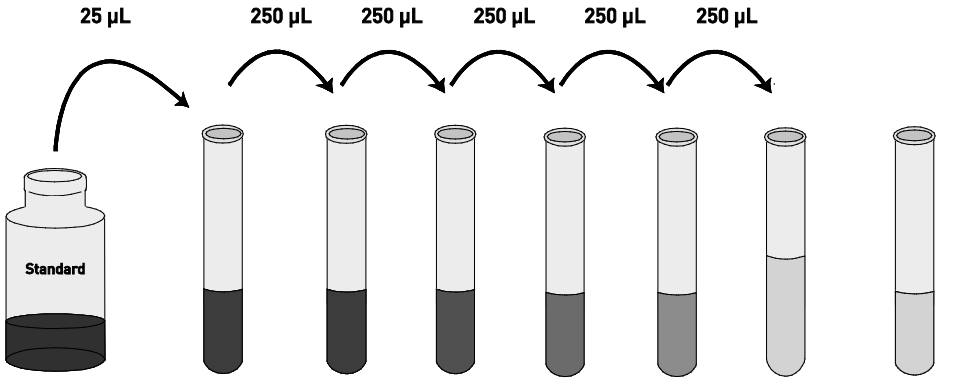
1. Dilute cell lysate samples by adding 4 volumes of Assay Buffer (the SSA concentration will be 1%, with a sample dilution of 1:5).
2. Perform an additional dilution of ≥1:4 with Sample Diluent prior to the assay (for a final dilution of ≥1:20).

## Dilute standards

**Note:** Use glass or plastic tubes for diluting standards.

**Important: If measuring GSSG, dilute standards with Sample Diluent containing 2VP**:

1. Prepare Std 1.
   a. **To measure total GSH**, add 25 µL Oxidized Glutathione Standard to one tube containing 475 µL Sample Diluent and label as 25 µM total GSH.
   b. **To measure GSSG**, Add 1 µL of 2VP solution to 50 µL Oxidized Glutathione Standard and incubate for 1 hour at room temperature. Then add 25 µL of the treated standard to one tube containing 475 µL Sample Diluent and label as 12.5 µM GSSG.
2. Prepare standard dilution tubes.
   a. **To measure total GSH**, add 250 µL Sample Diluent to each of 6 tubes labeled as follows: 12.5, 6.25, 3.125, 1.56, 0.781, and 0 µM total GSH.
   b. **To measure GSSG**, add 250 µL Sample Diluent to each of 6 tubes labeled as follows: 6.25, 3.125, 1.56, 0.781, 0.391, and 0 µM GSSG.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.




| Diluent volume | 475 µL | 250 µL | 250 µL | 250 µL | 250 µL | 250 µL | 250 µL |
|---|---|---|---|---|---|---|---|
| | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | 0 Std |
| GSSG | 12.5 µM | 6.25 µM | 3.125 µM | 1.56 µM | 0.781 µM | 0.391 µM | 0 µM |
| Total GSH | 25 µM | 12.5 µM | 6.25 µM | 3.125 µM | 1.56 µM | 0.781 µM | 0 µM |

## Prepare Colorimetric Detection Reagent

Prepare Colorimetric Detection Reagent according to the following table.

**Note**: Detection Reagent Concentrate contains DMSO, an aprotic organic solvent shown to enhance the absorption rate of skin-permeable substances. Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.

| Reagent | ½ plate | 1 plate | 2 plates | 4 plates |
|---|---|---|---|---|
| Detection Reagent Concentrate | 140 µL | 260 µL | 500 µL | 1 mL |
| Assay Buffer | 1.26 mL | 2.34 mL | 4.5 mL | 9 mL |
| Total volume | 1.4 mL | 2.6 mL | 5 mL | 10 mL |

## Prepare Reaction Mixture

1. Vortex the vials of Glutathione Reductase Concentrate and NADPH Concentrate.
2. Prepare Reaction Mixture according to the following table and vortex thoroughly.

| Reagent | ½ plate | 1 plate | 2 plates | 4 plates |
|---|---|---|---|---|
| NADPH Concentrate | 140 µL | 260 µL | 500 µL | 1 mL |
| Glutathione Reductase Concentrate | 140 µL | 260 µL | 500 µL | 1 mL |
| Assay Buffer | 1.12 mL | 2.08 mL | 4 mL | 8 mL |
| Total volume | 1.4 mL | 2.6 mL | 5 mL | 10 mL |

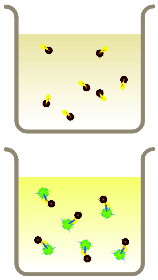
3. Store any unused Reaction Mixture at 4°C in an amber vial for no more than 2 days.

## Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

**IMPORTANT!** Perform a standard curve with each assay.

- **To measure GSSG**, assay 2VP treated standards, and samples.



### Add sample and detection reagent

a. Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.
b. Add 25 µL Colorimetric Detection Reagent into each well.



### Add reaction mixture

a. Add 25 µL Reaction Mixture into each well.
b. Tap the sides of the plate and mix.
c. Incubate for 20 minutes at room temperature. [1]


[1] To perform a kinetic assay, do not incubate. Read the absorbance immediately, and continue to take readings at 1 minute intervals for at least 10 minutes.

## Read the plate and generate the standard curve

1. Read the absorbance at 405 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   **Note**: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

## Guidelines for calculating glutathione concentration

- The concentration of oxidized glutathione (GSSG) is half of the GSH concentration read from the curve (i.e., 1 GSSG = 2 GSH).
- Free glutathione (GSH) concentrations are determined by subtracting the GSSG concentration from values obtained from non-treated samples and standards.
- Concentrations are expressed in µM of glutathione.

## Performance characteristics

### Standard curve (example)

The following data were obtained for the various standards.

| Standard Glutathione (µM) | GSSG | Total GSH |
|---|---|---|
| | Optical Density (405 nm) | Optical Density (405 nm) |
| 25 | — | 1.239 |
| 12.5 | 1.086 | 0.673 |
| 6.25 | 0.619 | 0.368 |
| 3.125 | 0.364 | 0.224 |
| 1.56 | 0.222 | 0.155 |
| 0.781 | 0.156 | 0.123 |
| 0.391 | 0.117 | — |
| 0 | 0.087 | 0.086 |

### Intra-assay precision

Whole blood and urine samples were assayed for total GSH in replicates of 20 to determine precision within an assay.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (µM) | 9.36 | 5.55 | 3.30 |
| %CV | 2.1 | 3.1 | 5.0 |

CV = Coefficient of Variation

### Inter-assay precision

Whole blood and urine samples were assayed for total GSH 20 times in duplicate by four operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (µM) | 9.43 | 5.27 | 3.08 |
| %CV | 7.5 | 8.4 | 13.3 |

CV = Coefficient of Variation

### Linearity of dilution

Linearity was determined by assaying high and low concentration cell lysates (high sample $25 \times 10^6$ cells/mL; low sample $1.28 \times 10^6$ cells/mL) mixed in the ratios shown in the following table.

| Low Sample % | High Sample % | Expected Conc. (µM) | Observed Conc. (µM) | % Recovery |
|---|---|---|---|---|
| 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 |
| | | | **Mean Recovery** | **103.7%** |

### Sensitivity

The analytical sensitivity of the assay is 0.634 µM glutathione. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## Limited product warranty

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**Manufacturer's address**: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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