
Product Manual

Total Thiol Assay Kit (Colorimetric)

Catalog Number

MET- 5053

192 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.

Creating Solutions for Life Science Research

Introduction

A thiol is a compound that contains a carbon-bonded sulfhydryl ($-C-SH$) group. Natural thiols include compounds such as glutathione, cysteine, and homocysteine. Thiols represent the largest part of the overall antioxidant pool in animals, and thiols therefore play a major role in protecting against damage from reactive oxygen species. Thiols can exist in the free reduced state or in covalent disulfide bridges with other molecules or attached to proteins through the sulfhydryl group of the thiol amino acid cysteine. Although thiols can bind various proteins, most protein bound thiols are attached to serum albumin at cysteine 34.

In addition to their role protecting against free radical stress, thiols are important in apoptosis, cell signaling, and cell detoxification. Nitric oxide effects in the body are controlled by S-nitrosylation of proteins and/or peptides to produce S-nitrosothiols (SNOs). Evidence suggests that inability to efficiently process SNOs is partly responsible for hypertension observed in preeclampsia. Chronic renal failure has been correlated with overall lower thiol status in the kidney. Oxidative stress has been suggested to contribute to diabetes pathogenesis, and patients who suffer from complications from type 2 diabetes mellitus also have lower protein thiol levels in serum.

Assay Principle

Cell Biolabs' Total Thiol Assay Kit provides a convenient colorimetric method for the detection of free sulfhydryl groups from cell lysates, tissue, plasma, serum, saliva or urine. First, the unknown samples or standards are added to a 96 well plate. Then, a Colorimetric Probe is added to the well which covalently reacts with the sulfhydryl to release a chromophore and the absorbance of the plate is read at 450 nm. The content of thiol in the unknown samples is determined by comparison with a predetermined reduced glutathione standard curve. The provided reagents are sufficient for the evaluation of 192 assays* including standards and unknown samples.

**Note: Each sample replicate requires 2 assays, one including the Colorimetric Probe and one without. Total thiol concentration is calculated from the difference in OD readings from the 2 wells.*

Related Products

1. STA-312: Total Glutathione Assay Kit
2. STA-670: Homocysteine ELISA Kit
3. STA-671: S-Adenosylhomocysteine (SAH) ELISA Kit
4. STA-672: S-Adosylmethionine (SAM) ELISA Kit
5. STA-675: Hydroxyproline Assay Kit

Kit Components

1. Reduced Glutathione Standard (Part No. 50531D): One 100 μ L vial containing 100 mM Reduced Glutathione
2. 10X Assay Buffer (Part No. 50532A): Four 2 mL vials
3. Colorimetric Probe (50X) (Part No. 50533C): One 400 μ L vial

Materials Not Supplied

1. 96 well ELISA strips or 96 well microtiter plate
2. 0.6 mL or 1.5 mL microcentrifuge tubes
3. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
4. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm

Storage

Upon receipt, store the entire kit at -20°C.

Preparation of Reagents

- 1X Assay Buffer: Combine all four tubes of 10X Assay Buffer to yield 8 mL. Dilute the 10X Assay Buffer 1:10 with deionized water (72 mL) to make 80 mL of a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- 1X Colorimetric Probe: Prepare the 1X Colorimetric Probe just before use. Prepare only enough for immediate applications. Dilute the Colorimetric Probe (50X) stock 1:50 with 1X Assay Buffer (e.g. Add 20 μ L of Colorimetric Probe (50X) stock to 980 μ L of 1X Assay Buffer). Vortex thoroughly.

Preparation of Standard Curve

Prepare a dilution series of Reduced Glutathione standards in the concentration range of 0 to 1.0 mM by diluting the Reduced Glutathione Standard in 1X Assay Buffer (Table 1).

Standard Tubes	100 mM Reduced Glutathione Standard (μL)	1X Assay Buffer (μL)	Reduced Glutathione (μM)
1	5	495	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 1. Preparation of Reduced Glutathione Standards.

Preparation of Samples

These preparation protocols are intended as a guide for preparing known samples. The user may need to adjust how samples are treated accordingly. All samples should be assayed immediately or store at -80°C for up to 1-2 months. A trial assay with a representative test sample should be assayed to determine the samples compatibility with the dynamic range of the standard. It is recommended that samples be processed as soon as possible because thiols are rapidly metabolized and will continue to form disulfides. The assay can be used on cell culture supernatants and lysates, plasma, serum, urine, saliva, tissue homogenates as well as other biological fluids. High levels of interfering substances may cause variations in results. Run proper controls as necessary. Always run a standard curve with samples.

Notes: Exogenously added thiol compounds, such as cysteine, dithiothreitol (DTT), or β -mercaptoethanol can interfere with the assay by competing with sample thiols for binding to the Colorimetric Probe. In addition, N-ethylmaleimide or other thiol alkylating reagents should also be avoided because they will interfere with thiols.

- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Dilute as necessary into 1X Assay Buffer. Store on ice if assaying immediately or freeze at -80°C for future use.
- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Dilute as necessary into 1X Assay buffer. Store on ice if assaying immediately or freeze at -80°C for future use.
- Saliva or Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary into 1X Assay Buffer. Store on ice if assaying immediately or freeze at -80°C for future use.
- Cell Lysate: Detach adherent cells by trypsinization. Count cells and centrifuge at 500 rpm for 5 minutes at 4°C. Wash cells with cold 1X PBS. Centrifuge suspension cells at 500 rpm for 5 minutes at 4°C. Remove supernatant and resuspend cells with cold 1X PBS. Homogenize or sonicate cell suspension and store on ice until use. Transfer the suspension to a microfuge tube and centrifuge at 12,000 rpm for 5 minutes at 4°C. Collect the supernatant. The supernatant may be assayed directly or diluted as necessary into 1X Assay buffer. Store on ice if used immediately or freeze at -80°C for future use.
- Tissue Lysate: The total thiol concentration in most tissues is in the millimolar range. It is recommended that a 10% w/v homogenate be created. Blood can contaminate a tissue sample due to high thiol concentrations. Therefore, perfusion of the tissue with a PBS/heparin is recommended. Remove tissue and wash the tissue thoroughly with cold isotonic saline solution of 1X PBS with 0.16 mg/mL heparin to prevent coagulation. Blot the tissue dry and weigh. Add cold 1X PBS (~1 mL/100 mg tissue) and homogenize using a glass pestle. Centrifuge the homogenate at 12,000 rpm for 15 minutes at 4°C. Collect the supernatant. Assay the supernatant directly or

diluted as necessary into 1X Assay buffer. Store on ice if used immediately or freeze at -80°C for future use.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each control, unknown sample and standard should be assayed in duplicate.

Note: Each control or unknown sample replicate requires two paired wells, one to be treated with Colorimetric Probe and one without Colorimetric Probe as described below.

2. Add 100 μ L of unknown samples or standards to a 96 well microplate.
3. Add 100 μ L of 1X Colorimetric Probe to the standard wells or one half of the paired unknown sample wells and mix the well contents thoroughly.
4. Add 100 μ L of 1X Assay Buffer to the other half of the paired unknown sample wells and mix thoroughly.
5. Incubate for 15 minutes at room temperature on an orbital shaker.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical Total Thiol Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

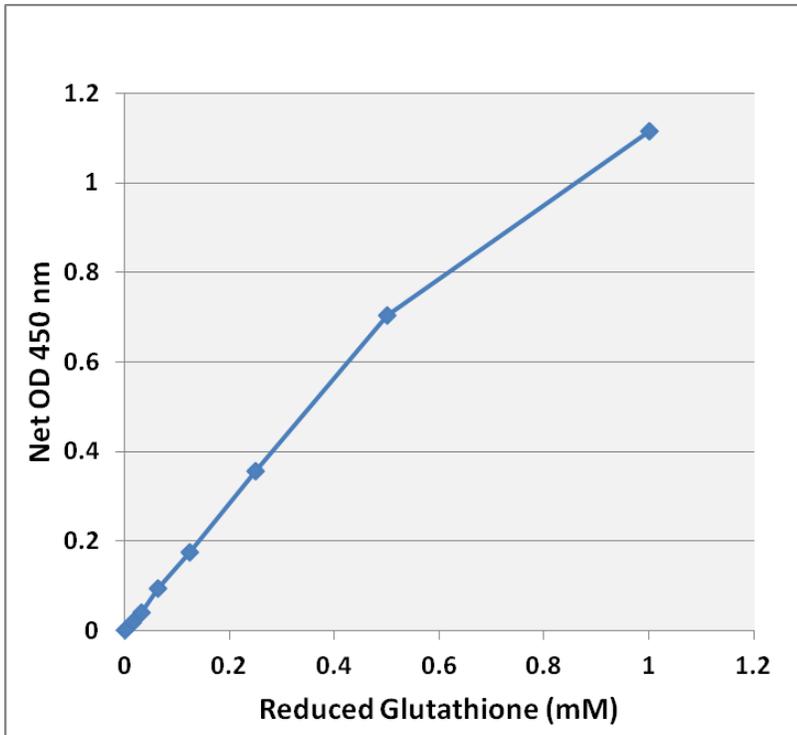


Figure 1: Reduced Glutathione standard curve.

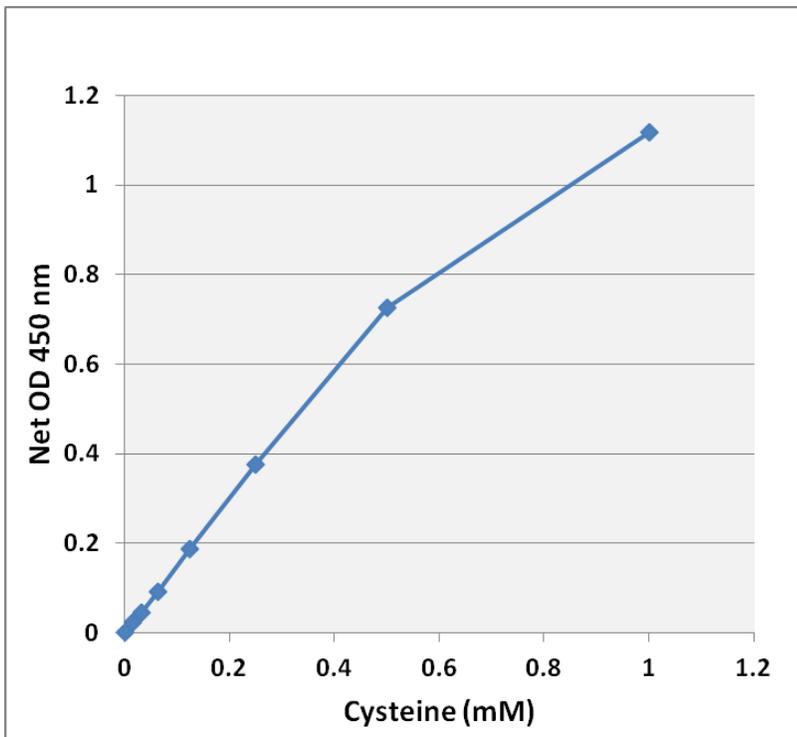


Figure 2: Detection of an example thiol. Purified Cysteine was resuspended in 1X assay buffer at various concentrations. Samples were tested according to the Assay Protocol.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 1).
4. Subtract the unknown sample well values without 1X Colorimetric Probe from the unknown sample well values containing 1X Colorimetric Probe to obtain the difference. The absorbance difference (ΔA) is due to total thiol interaction with 1X Colorimetric Probe:

$$\Delta A = A_{\text{Probe}} - A_{\text{1X Assay Buffer}}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of total thiols present in the sample. Only use values within the range of the standard curve.

References

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Warranty

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