Thiol (total) Assay Kit (Colorimetric)

Thiol (total) Assay Kit (Colorimetric) is a detection kit for the quantification of Thiol (total) in serum, plasma, saliva, urine, cell lysate and tissue lysate.

Catalog number: ARG82239

Package: 192 assays

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INTRODUCTION

A thiol or thiol derivative is any organosulfur compound of the form R–SH, where R represents an alkyl or other organic substituent. The –SH functional group itself is referred to as either a thiol group or a sulfhydryl group, or a sulfanyl group. Thiols are the sulfur analogue of alcohols (that is, sulfur takes the place of oxygen in the hydroxyl group of an alcohol), and the word is a blend of "thio-" with "alcohol", where the first word deriving from Greek θεῖον (theion) meaning "sulfur".

Many thiols have strong odors resembling that of garlic or rotten eggs. Thiols are used as odorants to assist in the detection of natural gas (which in pure form is odorless), and the "smell of natural gas" is due to the smell of the thiol used as the odorant. Thiols are sometimes referred to as mercaptans. The term "mercaptan" was introduced in 1832 by William Christopher Zeise and is derived from the Latin mercurio captāns (capturing mercury) because the thiolate group (RS–) bonds very strongly with mercury compounds. [Provide by Wikipedia: Thiol]

PRINCIPLE OF THE ASSAY

This Thiol (total) Assay Kit (Colorimetric) is a simple colorimetric assay that measures the total amount of free sulfhydryl group present in cell lysates, tissue, plasma, serum, saliva or urine. The assay is based on an enzyme driven reaction: First, the samples or standards are added into 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 concentration of thiol in the samples is then determined by comparing the 450 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store the entire kit at -20°C. Avoid multiple freeze/thaw cycles.

Component	Quantity	Storage information
Reduced Glutathione Standards (100 mM)	100 µL	-20°C
10X Assay Buffer	4 x 2 mL	-20°C
50X Colorimetric Probe	400 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate Reader capable of reading at 450 nm
- Deionized or Distilled water
- 1X PBS
- 96-well microplate or 96 well ELISA strip
- Microcentrifuge tubes (0.6 mL or 1.5 mL)
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store the entire kit at -20°C. Avoid multiple freeze/thaw cycles.
- Each sample replicate requires 2 assays, one including the Colorimetric Probe and one without. Total thiol concentration is calculated from the difference from the 2 wells.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue Lysates: Sonicate or homogenize tissue sample in cold 1X PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold 1X PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2,000 x g and 4°C for 10 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Urine: To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.
3. It is recommended that samples be processed as soon as possible because thiols are rapidly metabolized and will continue to form disulfides.

4. All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.
5. 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.

REAGENT PREPARATION

- 1X Assay Buffer: Combine all four tubes of 10X Assay Buffer to yield 8 mL. Dilute the 10X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 8 mL of 1X Assay Buffer into 72 mL of deionized water to a final volume of 80 mL) Stir or vortex to homogeneity. Store at room temperature.
- 1X Colorimetric Probe: Dilute the 50X Colorimetric Probe into 1X Assay Buffer to yield 1X Colorimetric Probe. (E.g., add 20 µL of 50X Colorimetric Probe into 980 µL of 1X Assay Buffer to a final volume of 1 mL) Stir or vortex to homogeneity.

  Note: Prepare the 1X Colorimetric Probe just before use. Prepare only enough for immediate applications.

- **Standards:** Prepare fresh Reduced Glutathione Standards by diluting in 1X Assay Buffer according to the Table below.

Standard tubes	Final Reduced Glutathione conc. (µM)	Volume of 1X Assay Buffer (µL)	Volume of 100 mM Reduced Glutathione Standard (µL)
S1	1000	495	5
S2	500	250	250 of S1
S3	250	250	250 of S2
S4	125	250	250 of S3
S5	62.5	250	250 of S4
S6	31.3	250	250 of S5
S7	15.6	250	250 of S6
S0	0	250	0

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

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

1. Add **100 µL** of **samples** or serial **diluted Reduced Glutathione Standards** into 96-well microplate.
2. Add **100 µL** of **1X Colorimetric Probe** into the standard wells or one half of the paired sample wells and mix the well contents thoroughly.
3. Add **100 µL** of **1X Assay Buffer** to the other half of the paired sample wells and mix thoroughly.
4. Incubate for **15 minutes** at **room temperature** on a microplate shaker.

5. Read the plate at a **wavelength of 450 nm** with a microplate reader.

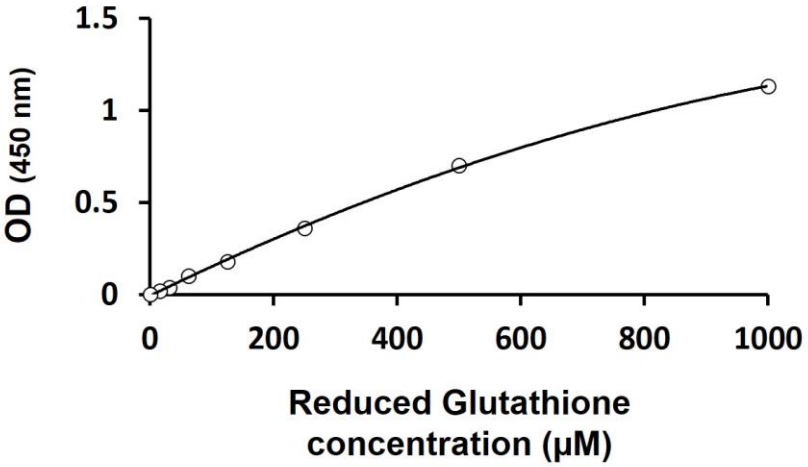
CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average Standard 0 value from itself and all standard values.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Subtract the sample well values without 1X Colorimetric Probe from the 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.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

16 µM