Thiol Assay Kit

Catalog No.: abx298894

Size: 96 tests (40 samples)

Detection Range: 9.91 µmol/L - 1000 µmol/L

Sensitivity: 9.91 µmol/L

Storage: Store all components at 2 – 8°C for up to 6 months.

Application: For detection and quantification of Thiol concentration in serum, plasma, tissue and other biological fluids.

Introduction

Thiol groups (sulfhydryl groups), found in free cysteine, glutathione (GSH), and cysteine residues in proteins, are involved in many biological processes. The disulfide bonds generated when the thiol groups of two cysteine residues are oxidized contribute to the tertiary or quaternary structure of a protein.

Abbexa's Thiol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Thiol concentration. Sulfyydryl compounds reacts with NBA (2-nitrobenzoic acid) under neutral or alkaline conditions to produce yellow color product and have absorption maxima at 412 nm. The absorbance should be measured at 412 nm. The intensity of the color is proportional to the Thiol concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer solution: 20 ml
3. Chromogenic agent: 1.3 ml
4. Standard: 2 vials
5. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (412 nm)
2. Double distilled water
3. Absolute ethyl alcohol (AR)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or Sonicating water bath
7. Centrifuge
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml Normal saline. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human plasma	4-6
Human serum	4-6
Human urine	1
10% Rat liver tissue homogenate	4-6
Rabbit serum	3-5
Porcine serum	4-6
10% Rat kidney tissue homogenate	4-6
10% Rat spleen tissue homogenate	4-6

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **5 mmol/L standard solution:** Dissolve a vial of standard with 10 ml of normal saline. Prepare immediately before carrying out the assay and mix fully. Unused Substrate working solution can be stored at 2-8°C for a day.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mmol/L. Dilute the 5 mmol/L standard solution with normal saline to concentrations of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mmol/L. The normal saline itself serves as the 0 mmol/L (blank) standard.

2. Chromogenic Reaction:

2.1. Set the Standard, Sample and Control wells.
2.2. Add 40 µl of prepared standards to the Standard wells.
2.3. Add 40 µl of samples to the Sample wells.
2.4. Add 40 µl of samples to the Control wells.
2.5. Add 150 µl of Buffer solution to each well.
2.6. Add 10 µl of Chromogenic agent to Standard and Sample wells.
2.7. Tap the plate gently to mix. Allow to stand at room temperature for 10 minutes.
2.8. Measure the OD values at 412 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{412} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The Thiol concentration of the samples can be interpolated from the standard curve.

1. Serum and plasma samples:

$$\text{Total Thiol } (\mu\text{mol/L}) = \frac{\Delta A_{412} - b}{a} \times 1000^{*} \times f$$

2. Tissues samples:

$$\text{Total Thiol } (\mu\text{mol/g tissue weight}) = \frac{\frac{\Delta A_{412} - b}{a}}{\frac{m}{V}} \times f$$

where:

ΔA_{412}	OD value of the sample ($OD_{Sample} - OD_{Control}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
V	volume of normal saline for preparation of tissue sample, ml
m	fresh weight of sample, g
1000^{*}	1 mmol/L=1000 µmol/L