Total Glutathione Assay (TGA)

Cat. No. 8518
100Tests in 96-well plate

Introduction

Reduced glutathione (GSH) is a tripeptide (g-glutamylcysteinylglycine) and plays a key role in preventing oxidative damage in living organisms. GSH is commonly the most abundant low molecular mass thiol in living cells. Intracellular GSH status appears to be a sensitive indicator of the redox state of a cell. High levels of GSH have been proven to correlate with good health and longevity, whereas low levels of GSH are believed to contribute to many medical conditions, such as heart attacks and neurodegenerative diseases. This colorimetric assay is based on the reaction between 5', 5'-Dithiobis 2-nitrobenzoic acid (DTNB) and GSH to form TNB, which exhibits maximum absorbance at 412 nm. The intensity of the absorbance is proportional to the GSH level in the sample. Since glutathione reductase is used in this assay, the level of GSH in the sample reflects the total glutathione (GSSG+GSH) level.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8518a	1	SSA powder	2.5 g	4°C
8518b	1	Assay buffer	25mL	4°C
8518c	1	Glutathione standard	20µL	-20°C
8518d	1	DTNB	1.0 mL	-20°C
8518e	1	NADPH	25 µL	-20°C
8518f	1	Enzyme	20 µL	-20°C

Product Use

The Total Glutathione Assay kit measures the total glutathione level of different types of samples, such as plasma, tissues and cell lysate. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from the Total GlutathioneAssay kit measuring glutathione solutions with concentrations ranging from 3.125 to50 µM show a linear relationship between OD_{412nm} and total glutathione concentration (Figure 1).

Shipping

Shipped on dry ice.

Reagents Preparation

1. SSA solution: Dissolve the contents of the bottle of SSA powder (8518a) in 50 ml of water and keep at 4°C.
2. Diluted glutathione standard: Add 2 µL glutathione standard (8518c) into 198 µL SSA solution.
3. Diluted enzyme solution: Add 2 µL enzyme (8518f) into 130 µL assay buffer (8518b).
4. Diluted NADPH solution (enough for 25 tests): Add 5 µL NADPH (8518e) into 1250 µL assay buffer (8518b).
5. Working mixture (enough for 25 tests): Add 114 µL of diluted enzyme solution and 114 µL of DTNB (8518d) into 4 mL assay buffer (8518b).

Procedure (96-well plate)

A. Preparation of glutathione standard

1. Obtain 6 test tubes and label them #1 through #6, add 110 µL of SSA solution into #1-6tube.
2. Add 110 µL diluted glutathione standard into tube #1, mix well in tube #1 to get the 50 µM glutathione standard.
3. Transfer 110 µL of the 50 µM glutathione standard from tube #1 to tube #2 and mix well to get the 25 µM glutathione standard.
4. Repeat step 3 for tubes #3-5 to serially dilute the glutathione standards. Do not add any glutathione to tube #6, which serves as the blank.
5. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each glutathione standard by aliquoting50 µL/well of each glutathione standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6
A	50 µM	25 µM	12.5 µM	6.25 µM	3.125 µM	Blank
B	50 µM	25 µM	12.5 µM	6.25 µM	3.125 µM	Blank

B. Preparation of test samples

1. Cell pellets or tissues can be homogenized in 4 volumes of the SSA solution. Keep on ice for 5 minutes and centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble, clear fraction can be assayed directly. The sample can be stored at -80ºC for one month. For plasma, mix 100 µL plasma with 100 µL SSA solution, keep on ice for 5 minutes, and centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to get the supernatant.
2. Samples should be serial diluted to make sure the readings are within the standard curve range.Prepare test samples to a final volume of 50 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. Prepare appropriate volume of glutathione assay working reagent based on the number of samples to be measured. For each well of reaction, prepare 150µL working mixture and 50µL diluted NADPH solution.
2. Add 150 µL of working mixture into each well of the 96-well plate containing glutathione standard, samples and blank, mix well and wait for 5 minutes. Add 50µL diluted NADPH solution into each well of the 96-well plate containing glutathione standard, samples and blank. Start recording OD_{412nm} over a 5 minutes interval, collecting data every 1 minute.

D. Calculations

1. Subtract the measured OD_{412nm} at different reaction time from the initial OD_{412nm} to obtain the corresponding ΔOD_{412nm} for each sample and glutathione standard at different reaction time. Average the value of ΔOD_{412nm} of replicate wells.
2. Based on the ΔOD_{412nm} of the glutathione standard solutions, plotting the absorbance at ΔOD_{412nm} as a function of reaction time (Figure 1) in which ΔOD_{412nm} /min is calculated. Subtract the measured ΔOD_{412nm} /min at different reaction time from the blank.
3. Plot a standard curve of ΔOD_{412nm} /min vs glutathione standard solutions (Figure 2).
4. Calculate the glutathione level of test samples based on the standard curve.
5. Suppose the equation of the trend line of standard curve is $y=Ax+B$,

   Calculate the total glutathione concentration of test samples as follows:

$$[GSH_{total}] = 2 \times \frac{\Delta OD_{412nm}/min - B}{A} \times \text{sample dilution}$$

   Note: Sample dilution is 1 for tissue and cells sample and 2 for plasma sample.

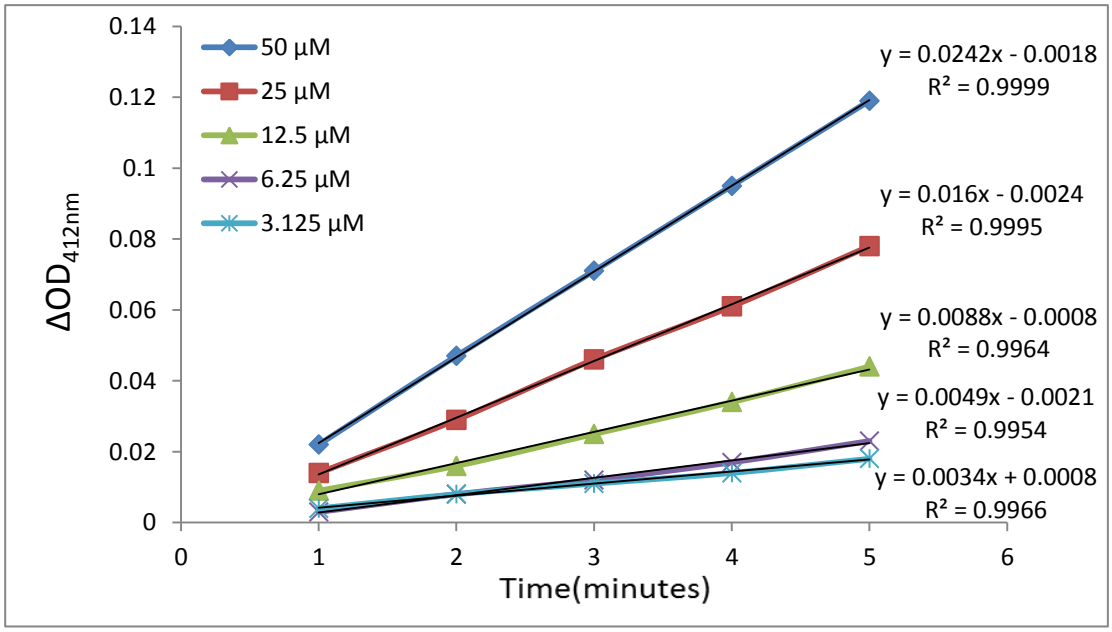

Figure1. Standard curves of ΔOD_{412nm} vs reaction time for glutathione solution with different activity.

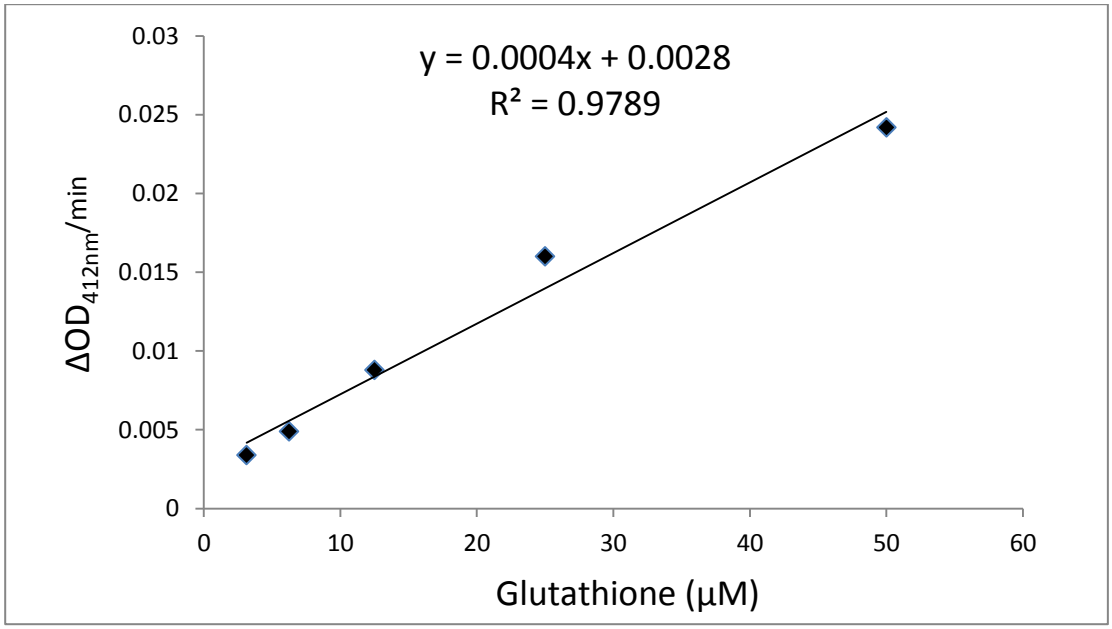

Figure2. Standard curve of ΔOD_{412nm}/min vs concentration of glutathione. TheΔOD_{412nm}/min is calculated as the slope of the standard curves shown in Figure 1.

References

1. Akerboom TP, and Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol 77, 373-382 (1981).
2. Eyer P, and Podhradsky D. Evaluation of the micromethod for determination of glutathione using enzymatic cycling and ELLman's reagent. Anal Biochem 153, 57-66 (1986).