GSH/GSSG Ratio Assay (GSH/GSSG)

Cat. No. 8558
100Tests in 96-well plate

Introduction

Glutathione is a tripeptide (g-glutamylcysteinylglycine) and exists in reduced (GSH) and oxidized (GSSG) states. Glutathione peroxidase catalyzes the formation of GSSG from GSH, and glutathione reductase recycles GSSG to GSH. With increased levels of oxidative stress, intracellular GSSG will accumulate, and the GSH/GSSG ratio will decrease. Therefore, the determination of the GSH/GSSG ratio is a useful indicator of oxidative stress in cells and tissues. 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.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8558a	1	SSA powder	2.5 g	4°C
8558b	1	Dilution buffer	25mL	4°C
8558c	1	Assay buffer	25 mL	4°C
8558d	1	GSSG standard	25 µL	-20°C
8558e	1	Scavenger	0.1 mL	-20°C
8558f	1	DTNB	1.2mL	-20°C
8558g	1	NADPH	0.1 mL	-20°C
8558h	1	Enzyme	0.1 mL	-20°C

Product Use

The GSH/GSSG Ratio Assay kit measures the ratio between GSH and GSSG in different types of samples, such as tissues, cell lysate and plasma. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from the GSH/GSSG Ratio Assay kit measuring GSSG solutions with concentrations ranging from 0.195 to 6.25 µM show a linear relationship between OD_{412nm} and GSSG concentration (Figure 1). Linear detection range is 0.05 µM to 6.25 µM in 96-well plate assay.

Shipping

Shipped on dry ice.

Reagents Preparation

1. SSA solution: Dissolve the contents of the bottle of SSA powder (8558a) in 50 ml of water and keep at 4°C.
2. Diluted GSSG standard: Add 1 µL GSSG standard (8558d) into 399 µL SSA solution.
3. Diluted enzyme solution: Add 19 µL enzyme (8558h) into 125 µL assay buffer (8558c).
4. Diluted NADPH solution (enough for 25 tests): Add 25 µL NADPH (8558g) into 1250 µL assay buffer (8558c).
5. Working mixture (enough for 25 tests): Add 114 µL of diluted enzyme solution and 300 µL of DTNB (8558f) into 4 mL assay buffer (8558c).

Procedure (96-well plate)

A. Preparation of GSSG standard

1. Add 60 µL diluted GSSG standard to 60 µL of dilution buffer (8558b) to make 120 µL solution of 12.5 µM GSSG standard.
2. Obtain 7 test tubes and label them #1 through #7. Add 110 µL of dilution solution into tubes #1-7.
3. Add 110 µLto 12.5µM GSSG into tube #1 and mix well to get the 6.25 µM GSSG standard.
4. Transfer 110 µL of the 6.25 µM GSSG standard from tube #1 to tube #2 and mix well to get the 3.125 µM GSSG standard.
5. Repeat step 3 for tubes #3-6 to serially dilute the GSSG standard. Do not add any GSSG to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each GSSG standard by aliquoting 50 µL/well of each GSSG standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	6.25 µM	3.125 µM	1.56 µM	0.78 µM	0.39 µM	0.195 µM	Blank
B	6.25 µM	3.125 µM	1.56 µM	0.78 µM	0.39 µM	0.195 µM	Blank

B. Preparation of test samples

1. Cell pellet or tissue 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 serially diluted to make sure the readings are within the standard curve range. Mix 10 µL sample with 90 µL dilution buffer (8558b), then load the final volume of 50 µL/well of each test sample into two wells on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. Prepare appropriate volume of working reagent based on the number of samples to be measured. For each

well of reaction, prepare 150 µL working mixture and 50 µL diluted NADPH solution.

2. Add 1 µL scavenger (8558e) into one well of test sample, which is for GSSG assay. The other well without scavenger is for total glutathione assay. Let it rest for 1 minute.
3. Add 150 µL of working mixture into each well of the 96-well plate containing GSSG standard. Test samples and blank; mix well. Let it rest for 2 minutes. Add 50 µL diluted NADPH solution into each well of the 96-well plate containing GSSG standard, samples and blank. Start recording OD_{412nm} over a 3 minutes interval, collecting data every 0.5 minute.

D. Calculations

1. Subtract the measured OD_{412nm} at a different reaction time from the initial OD_{412nm} to obtain the corresponding ΔOD_{412nm} for each sample and GSSG standard at a different reaction time. Average the value of ΔOD_{412nm} of replicate wells.
2. Based on the ΔOD_{412nm} of the GSSG standard solutions, plot the absorbance at ΔOD_{412nm} as a function of reaction time (Figure 1) to calculate ΔOD_{412nm} /min. Subtract the measured ΔOD_{412nm} /min at a different reaction time from the blank.
3. Plot a standard curve of ΔOD_{412nm} /min vs. GSSG standard solutions (Figure 2).
4. Calculate the total glutathione level and GSSG level of test samples based on the standard curve (Figure 2).
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: ΔOD_{412nm}/min is the value from the well without scavenger treatment. Sample dilution is 10 for tissue and cells sample and 20 for plasma sample.

   Calculate GSSG concentration of test samples as follows:

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

   Note: ΔOD_{412nm}/min is the value from the well with scavenger treatment. Sample dilution is 10 for tissue and cells sample and 20 for plasma sample.

6. GSH/GSSG ratio is:

$$[GSH/GSSG] = \frac{[GSH_{total}] - 2 \times [GSSG]}{[GSSG]}$$

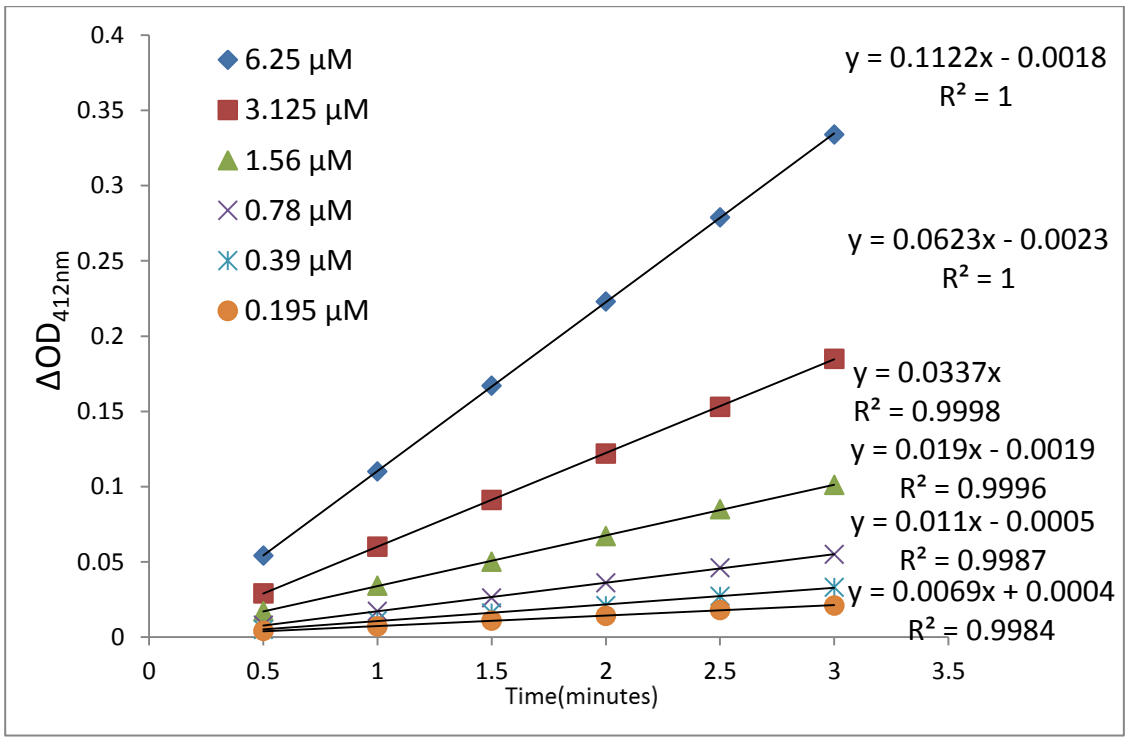

Figure 1. Standard curves of ΔOD_{412nm} vs. reaction time for GSSG solution with different activity.

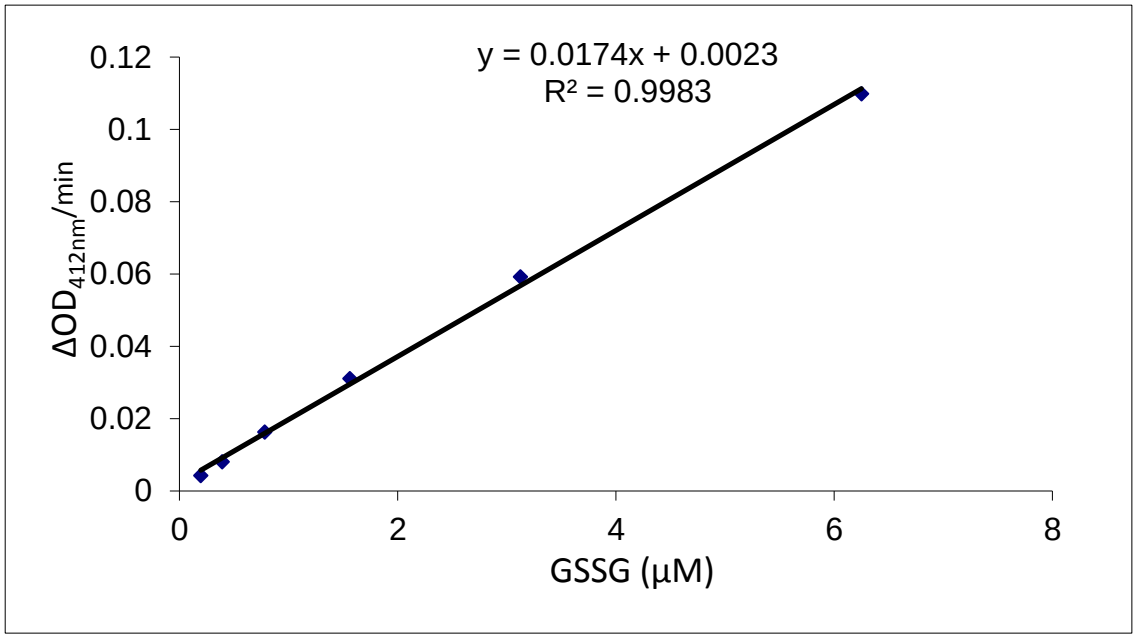

Figure 2. Standard curve of ΔOD_{412nm}/min vs. concentration of GSSG. The ΔOD_{412nm}/min is calculated as the slope of the standard curves shown in Figure 1.