

GENCHEM® Reduced Glutathione, GSH (Colorimetric) Assay Kit

REF : KBCA2066

Ver 1.0

RUO

Biochemical Assay for the Quantitative Determination of Reduced Glutathione, GSH (Colorimetric) in serum, plasma, Animal and Plant tissues, Red Blood cells, Cells, Bacteria

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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 96 tests

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Introduction:

Glutathione, GSH is a low molecular weight compound which can get rid of O_2^- , H_2O_2 & $LOOH$. GSH is a tripeptide composed by glutamic acid, glycine & cysteine, it is main non-protein sulfhydryl compound in tissue and substrate of GSH-PX & GST (GSH-PX & GST need GSH to decompose hydroperoxides). GSH can also stabilize sulfhydryl containing enzymes and avoid oxidative damage of hemoglobin & other cofactors. Recently, GSH is proved to participate function of recovering vitamin E to reduction form. Absence or exhausting of GSH will induce various chemicals or environmental factors to cause or aggravate toxication, it may relate to increasing oxidative damage, so GSH content is an important factor to measure oxidizing ability in vivo.

Intended Use

The GENCHEM® Reduced Glutathione, GSH (Colorimetric) Assay kit is used as an analytical tool for quantitative determination of Reduced Glutathione, GSH (Colorimetric) in serum, plasma, Animal and Plant tissues, Red Blood cells, Cells, Bacteria.

Principle:

Glutathione is a Tripeptide of Glycine, Glutamic acid and Cysteine. In the red blood cell, reduced Glutathione (GSH) is the key to maintain hemoglobin in reduced state and protect cells from Oxidative damage. GSH is the most important antioxidant sulfhydryl compound in cells, which plays an important role in oxidation resistance, protein sulfhydryl protection and amino acid transport across membrane. The ratio between reduced and oxidized Glutathione (GSH/GSSG) is the main indicator of the cell's redox state. Therefore, measuring the content of GSH and GSSG and the ratio of GSH/GSSG in cells can reflect the redox state of cells. This kit provides a simple method for detecting the content of GSH in a variety of biological samples, such as Serum, Plasma, Animal and Plant Tissues, Red Blood cells, Cells, Bacteria. DTNB reacts with reduced Glutathione to form a yellow product. The optical density measured at 412 nm, can directly reflect Glutathione concentration in the sample.

Materials Provided:

1. Extraction Buffer – 100 ml
2. Assay Buffer – 1 vial x 20 ml
3. Chromogen (Powder) – 1 vial
4. Standard (Powder) – 1 vial

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. After receiving the kit, an unopened kit can be stored at 4°C for 12 months, protected from light. Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

**Materials & Equipment Required But Not Provided**

1. Microplate Reader/Visible Spectrophotometer
2. Incubator
3. Refrigerated
4. Centrifuge
5. Ice maker
6. 96 well Plate
7. Pipettes

8. Deionized Water
9. Homogenizer (for Tissue Samples)

Sample Preparation

1. **Animal Tissues:** Weigh 0.1 g Tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. **Plant Tissues:** Weigh 0.1 g Tissues, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
3. **Blood Cells :** Collect blood using an anticoagulant. Centrifuge at 600 g for 10 min at 4°C. Discard the upper plasma, and then suspended with PBS. 5×10^6 blood cells were collected, washed twice with cold PBS (the blood cells were suspended with PBS, centrifuged at 600g at 4°C for 10min), add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 minutes at 4°C. Use supernatant for assay, and place it on ice to be tested.
4. **Cells or Bacteria:** Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 minutes at 4°C. Use supernatant for assay, and place it on ice to be tested.
5. **Plasma or Serum:** Serum was collected by routine method, add the same volume of Extraction Buffer. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested. Collect blood using an anticoagulant. Centrifuge at 600 g for 10 min at 4°C, take the upper plasma into another centrifuge tube within 30min, add the same volume of Extraction Buffer. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Reagent Preparation

1. **Extraction Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
2. **Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
3. **Chromogen:** Prepare before use, add 2.5 ml Deionized Water for 48T, 5 ml Deionized Water for 96T, mix well. Store at 4°C, protected from light.
4. **Standard preparation:**
Diluted Extraction Buffer: Make a 1:10 dilution of the Extraction Buffer solution with deionized water in a clean plastic tube by diluting 300 ul Extraction Buffer into 2,700 ul Deionized Water.
50 mM GSH standard: Each tube of GSH standard contains 15.4 mg GSH. Add 1ml Diluted Extraction Buffer to obtain 50 mM GSH standard. Store at 4°C, protected from light.
1000 uM GSH standard: Add 980 ul Diluted Extraction Buffer into 20 ul 50 mM GSH standard.
 Dilute the standard furtherly using 1000 uM GSH standard refer to the table below:

Standard Concentration	Standard Vial	Dilution Particulars
1000 uM	Standard No.7	200 ul Original Standard
500 uM	Standard No.6	100 ul Standard No.7 + 100 ul Diluted Extraction Buffer
250 uM	Standard No.5	100 ul Standard No.6 + 100 ul Diluted Extraction Buffer
125 uM	Standard No.4	100 ul Standard No.5 + 100 ul Diluted Extraction Buffer
62.5 uM	Standard No.3	100 ul Standard No.4 + 100 ul Diluted Extraction Buffer
31.25 uM	Standard No.2	100 ul Standard No.3 + 100 ul Diluted Extraction Buffer
15.6 uM	Standard No.1	100 ul Standard No.2 + 100 ul Diluted Extraction Buffer
0 uM	Standard No.0	100 ul Diluted Extraction Buffer only

Assay Procedure:

1. Preheat the Microplate Reader or Visible Spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, Visible Spectrophotometer deionized water zero.
2. Sample measurement (Add the following reagents respectively into the 96-well plate or microglass cuvette):

Reagent	Blank well (ul)	Standard well (ul)	Test well (ul)
Sample	0	0	20
Diluted Extraction Buffer	20	0	0
Standard	0	20	0
Assay Buffer	140	140	140
Chromogen	40	40	40

Mix well, Incubate for 10 minutes at 37°C, protected from light, record the absorbance value at 412 nm as A_{Blank} , A_{Standard} , and A_{Test} . Finally, calculate $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$, $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$. The Blank well only needs to measure 1 time.

Data Analysis**1. Drawing of standard curve**

With the concentration of the standard as the y-axis and the $\Delta A_{\text{Standard}}$ as the x-axis, draw the standard curve.

2. Calculate the GSH content of Sample

Substitute the ΔA_{Test} into the equation to obtain the y value ($\mu\text{M} = \text{nmol/mL}$).

Calculated by fresh weight of samples

$$\text{GSH (nmol/g fresh weigh)} = y \times V_{\text{Sample}} \div (W \times V_{\text{Sample}} + V_{\text{Sample Total}}) \times n = y \div W \times n$$

Calculated by liquid volume

$$\text{GSH (nmol/mL)} = 2 \times y \times V_{\text{Sample}} \div V_{\text{Sample}} \times n = 2y \times n$$

Calculated by Cells number

$$\text{GSH (nmol/10}^4 \text{ cells)} = y \times V_{\text{Sample}} \div (500 \times V_{\text{Sample}} + V_{\text{Sample Total}}) \times n = y \div 500 \times n = 0.002 \times y \times n$$

Where: V_{Sample} : Volume of supernatant added to the reaction system, 20ul= 0.02mL; W: sample mass, g; $V_{\text{Sample Total}}$: Volume of extraction solution, 1 mL; n: dilution multiple of further dilution of the sample; 2: dilution times when the liquid sample is prepared, adding the same volume of Extraction Buffer is diluted 2 times; 500: Number of cells, 5×10^6 .

Typical Data

Typical Standard Curve:

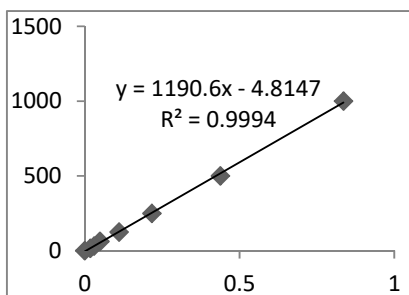


Figure1. Standard Curve of GSH in 96-well plate assay-data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Procedural Notes:

1. Wear appropriate personal protective equipment including lab coats, eye protection, and latex gloves. Adhere to all national security protocols for biological laboratories, particularly when handling blood samples or other bodily fluids.

2. Do not mix or use components from different lots. The kit must not be used beyond the expiration date printed on the kit label.
3. To avoid cross-contamination, change pipette tips between each reagent addition. Additionally, use separate reservoirs for each reagent.
4. Briefly centrifuge small vials at low speed before opening.
5. Fresh samples are recommended. If not assayed immediately, samples should be stored at -80°C for up to one month.
6. Always prepare fresh Standards for each use. Diluted Standard solutions are unstable and must be used within 4 hours of preparation.

Safety Precautions:

- **This kit is For Research Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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