(For Research Use Only. Not For Use in Diagnostic Procedures!)

GSH (Reduced Glutathione) colorimetric Assay Kit

Cat #: orb1952737 (manual)
Size: 48T/96T

Please do not mix and use reagents from different kits or different batches. Otherwise, it might not work properly.

Please read the manual carefully before use. Feel free to contact us if you have any questions.

Please provide the batch number (see kit label) for more rapid response and services.

It's strongly recommended to use this kit within the expiry date printed on the kit label.

Product Features

Application	In vitro quantitative determination of GSH concentrations in serum, plasma, cell culture supernatant and other biological samples.		
Reactivity	Universal	Detection Method	Colorimetric Assay
Range	1.56-100umol/L	Sensitivity	1.2umol/L
Detection Duration	20 mins (excluding sample preparation)		
Samples needed for single well (Max)	Serum: 10ul, Plasma: 10ul, Cell Culture Supernatant: 100ul, cell or tissue lysate: 100ul, Other liquid samples: 100ul		
Storage	2-8°C (for sealed box), please do not freeze! See kit label for expiry date		

Background

CAS: 70-18-8

Glutathione (GSH) is a tripeptide containing γ-amide bond and sulfhydryl group, which is composed of glutamate, cysteine and glycine, and is present in almost every cell of the body.
Glutathione can help maintain normal immune system function, and has antioxidant effects and integrated detoxification. The sulfhydryl group on cysteine is its active group (used to be abbreviated as G-SH), which is easy to combine with some drugs, toxins, etc., so that it has an integrated detoxification effect. Glutathione can not only be used in medicine, but also as the base material of functional food, which is widely used in delaying aging, enhancing immunity, anti-tumor and other functional foods.
There are two forms of glutathione, reduced (G-SH) and oxidized (G-S-S-G), with reduced glutathione accounting for the vast majority under physiological conditions. Glutathione reductase catalyzes the interconversion between the two forms, and its coenzyme also provides NADPH for pentose phosphate bypass metabolism. Quantification of GSH can be extended to drug discovery, pharmacology, toxicology studies and to study the effects of drugs and toxic compounds on glutathione metabolism.

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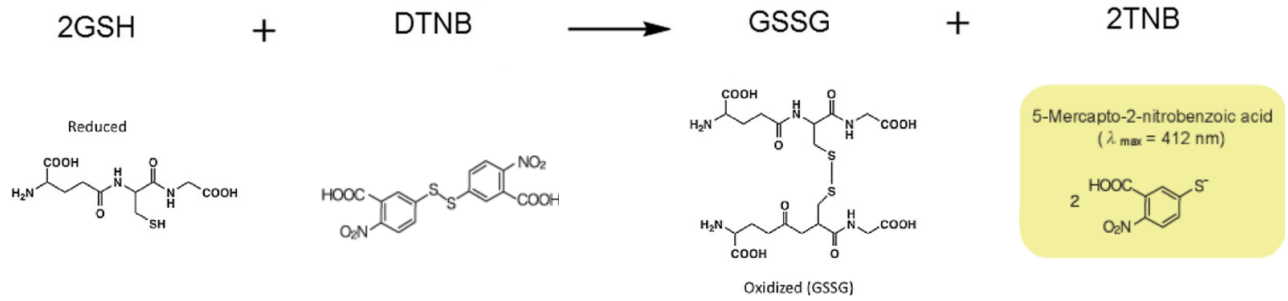
Principle of the Assay

The spectrophotometric/microplate reader assay method for glutathione (GSH) involves oxidation of GSH by the 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′ -thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. The lowest detection for GSH is 1.2umol in a 96-well plate. The concentration of GSH in the samples is determined by comparing the OD of the samples to the standard curve. The concentration of GSH is proportional to the OD value.

The kit is rapid and the whole procedure takes no longer than 20 min including reagent preparation. It can assay GSH in whole blood, plasma, serum, lung lavage fluid, cerebrospinal fluid, urine, tissues and cell.

Reaction mechanism:



Kit Components and Storage

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below:

Item	Size (48T)	Size (96T)	**Storage Condition**
ELISA Microplate	8×6	8×12	Stored for 6 months at 2-8°C
GSH Standard（3.07mg）	1vial	2 vials	
Standard stock solution	1.5ml	1.5mlx2	
DTNB (Concentrated, 20X)	270ul	520ul	
Assay Dilution Buffer	11ml	22ml	
Product Description	1 copy	1 copy	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

Required Instruments and Reagents

1. Microplate reader (wavelength: 405 or 414nm)
2. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)
3. Sterile tubes and Eppendorf tubes with disposable tips
4. 10mM PBS PH7.2-8.0

Sample Collection and Storage

1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. Plasma

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Sample

Tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces.

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for assay should be within 1-3mg/ml.

Notes: PBS buffer or the RIPA lysis can be used as lysates. Avoid using any reagents containing DTT Or reducing agent due to their severe interfere with kits' working.

4. Cell Culture Supernatant

Collect the supernatant: Centrifuge at 2500 rpm at 2-8°C for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

5. Cell Lysate

5.1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add pre-cooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1ml cell lysate. Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1ml cell lysate. Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes: Read notes in tissue sample.

6. Other Biological Sample

Centrifuge samples for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

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Notes for Samples

1. Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.

2. The best sample storage condition: less than 5 days at 2-8°C; within 6 months at -20°C; within 2 years at -80°C. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25°C can decrease the effect of ice crystal (0°C) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.

3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.

4. Pretest is recommended for special samples without reference data to validate the validity.

Precautions for Kits

1. After opening the kit, please refer to the table of storage condition for GSH standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace. (e.g. GSH standard)

2. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.

3. Before confirmation, reagents from other batches or sources should not be used in this kit.

4. Don't reuse tips and tubes to avoid cross contamination.

5. Please wear the lab coat, mask and gloves to protect yourself, during the assay. Especially, for the detection of blood or other body fluid sample, please follow regulations on safety protection of biological laboratory.

Recommended Sample Dilution Ratio

Random individual test data:

Sample Type	Recommended Dilution Ratio	Content
Human serum (Healthy)	1/10-1/20	700umol/L
Human plasma (Healthy)	1/10-1/20	625umol/L
Rat serum (Healthy)	1/10-1/20	708umol/L
Mouse serum (Healthy)	1/10-1/20	620umol/L
T47D cell lysate	1/5-1/10	252umol/2mg (total protein)
Human urine (Healthy)	undiluted	7.2umol/L

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2-fold dilution (1/2): One step dilution. Add 60µL sample into 60µL PBS and mix gently.

For 5-fold dilution (1/5): One step dilution. Add 24µL sample into 96µL PBS and mix gently.

For 10-fold dilution (1/10): One step dilution. Add 12µL sample into 108µL PBS and mix gently.

For 20-fold dilution (1/20): One step dilution. Add 6µL sample into 114µL PBS and mix gently.

For 50-fold dilution (1/50): One step dilution. Add 3µL sample and 47µL PBS into 100 µL PBS and mix gently.

For 100-fold dilution (1/100): One step dilution. Add 3µL sample and 177µL PBS into 120µL PBS and mix gently.

For 1000-fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first. Then, create a 20-fold dilution and mix gently.

For 10000-fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first. Then, create the same dilution again and mix gently.

For 100000-fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively. Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3µL. Dilution factor should be within 100-fold. Mixing during dilution is required to avoid foaming.

Reagent Preparation and Storage

The kit is ready for immediate use when taken out of the refrigerator.

1. 10mM GSH standard storage solution

1.1 Centrifuge GSH standards tube for 1min at 10000xg. Label it as 10mM GSH.

1.2 Add 1ml Standard stock solution buffer into the standard tube. Tighten the tube cap and invert the tube several times to mix gently. (Or you can mix it using a low-speed vortex mixer for 20 seconds.)

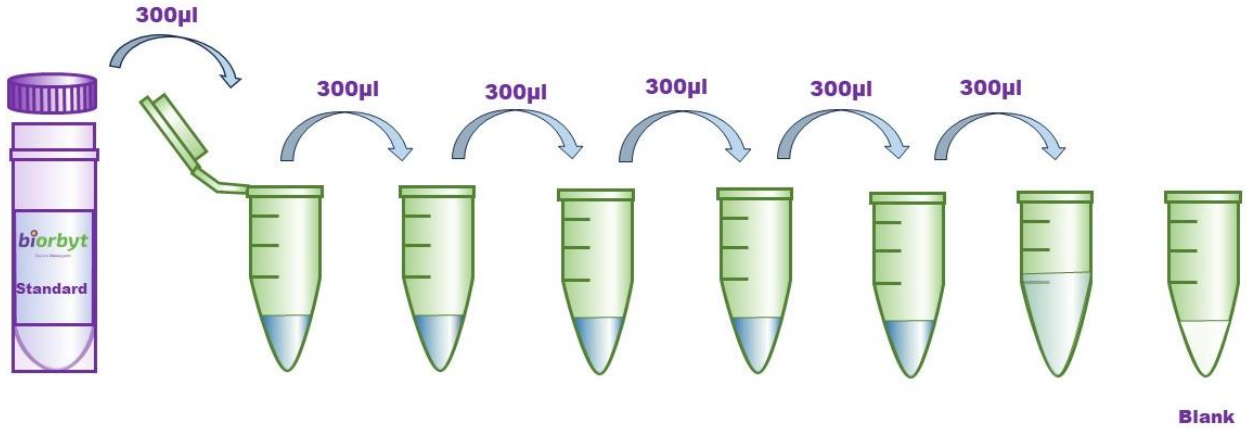
Centrifuge the tubes for 10s at 1000xg, making the liquid towards the bottom of tube.

2. Standards

2.1. Label an EP tube with zero tube. Add 990ul Assay Dilution Buffer and 10ul **10mM GSH standard storage solution** into it. Invert the tube several times to mix gently. (Or you can mix it using a low-speed vortex mixer for 3-5 seconds.)

2.2. Centrifuge the tubes for 1min at 1000xg, making the liquid towards the bottom of tube and removing possible bubbles.

2.3. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Assay Dilution Buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contains 0.3ml Assay Dilution Buffer. The standard concentration from zero tube to blank tube is 100umol/L, 50umol/L, 25umol/L, 12.5umol/L, 6.25umol/L, 3.125umol/L, 1.562umol/L, 0umol/L.



Notes: The 10mM GSH standard storage solution can be stored at -20 ° C for 6 months or at 2-8°C for 2 months. Other diluted working solutions containing standards should be used in 2h.

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3. Preparation of DTNB Working Solution

The working solution should be prepared within 5min before the assay and can't be stored for a long time.

3.1. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

3.2. Centrifuge for 10s at 1000xg in low-speed and bring down the concentrated DTNB (20X) to the bottom of tube.

3.3. Dilute the DTNB with Assay Dilution Buffer at 1/20 and mix them thoroughly. (e.g. Add 50ul concentrated DTNB into 950ul Assay Dilution Buffer.)

Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.

2. Standards and samples loading: Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube into each standard well. Also add Assay Dilution Buffer into the control (blank) well. Then, add 100ul pilot samples into each sample well.

3. DTNB Working Solution loading: Add 100ul DTNB Working Solution into each well. Then gently shake the plate to mix the working solution and incubated for 3 minutes at room temperature.

4. OD Measurement: Read the O.D. absorbance at 405nm or 414nm in a microplate reader immediately.

Calculation of Results

1. Calculate the mean OD value of the duplicate readings for each standard, control, and sample.

2. Create a Linear Fit curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4).

3. Calculate the sample concentration by substituting OD value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

sample type	IE: Linear Fit: y= a + bx (a=0.0819, b=0.0043)
Serum, plasma, CSF, urine	(GSH of sample umol/L) = (△A405 − a) ÷ b × f
Lysate: C=g/L (BCA method)	(GSH of sample umol/g) = (△A405 − a) ÷ b × f ÷ C

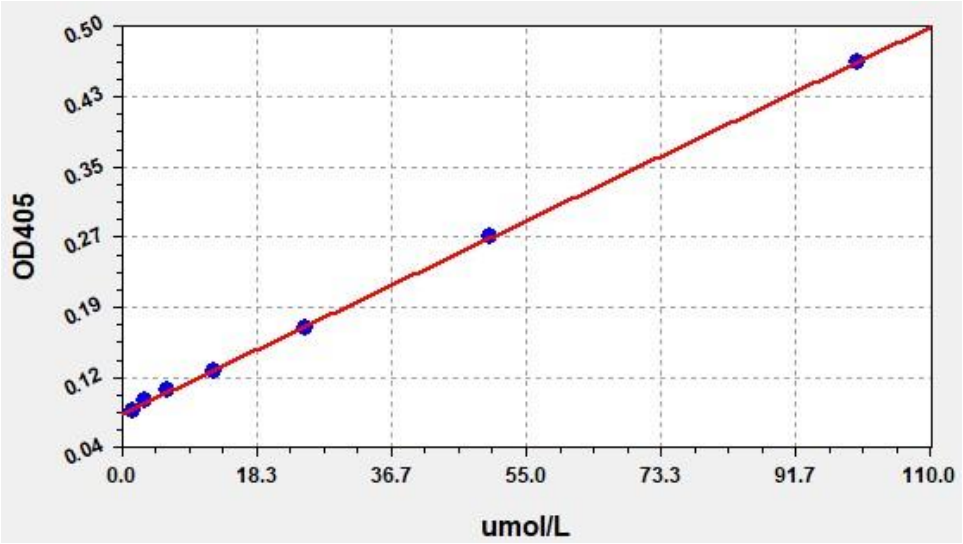
Note: f (sample dilution factor), C (total protein concentration of the samples)

Typical Data & Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C - 25°C.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD. (umol/L)	OD-1	OD-2	Average
0	0.079	0.08	0.08
1.562	0.083	0.083	0.083
3.125	0.092	0.092	0.092
6.25	0.103	0.104	0.104
12.5	0.125	0.126	0.126
25	0.172	0.174	0.173
50	0.272	0.275	0.274
100	0.464	0.462	0.463



Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (umol/L)	3	13.36	50.16	3.04	13.9	51.47
Standard deviation	0.13	0.57	2.35	0.16	0.77	3.25
CV (%)	4.23	4.23	4.68	5.22	5.54	6.32

Recovery

Add a certain amount of GSH into the sample. Calculate the recovery by comparing the measured value with the expected amount of GSH in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	88-100	95
EDTA Plasma (n=5)	88-103	97
Heparin Plasma (n=5)	86-102	95

Linearity

Dilute the sample with a certain amount of GSH at 1: 2, 1: 4 and 1: 8 to get the recovery range.

Sample	1: 2	1: 4	1: 8
Serum (n=5)	91-103%	90-101%	85-99%
EDTA Plasma (n=5)	95-104%	87-99%	83-95%
Heparin Plasma (n=5)	99-105%	89-101%	84-99%

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Troubleshooting

If the result is unsatisfied, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	The standard and DTNB working solution were incorrectly diluted	Confirm the required reagent added in each step. Also repeat the assay and verify.
	GSH standard is stored improperly, or expired	The GSH standards were re-prepared
	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Samples without signal	The amount of pilot sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.
	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the pilot sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline and regularly store.
	Longer storage of sample	Aliquot and store samples according to the assay requirement.
High CV%	The OD value of the sample exceeded the highest concentration of the standard curve.	Increase the dilution ratio of the sample.
	Unclean plate	Don't touch the bottom of the plate during the assay.
	Foam is found in the well.	Avoid foaming during reading in a microplate reader.
	Reagents are not completely mixed.	Mix all reagents completely.
	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the standard tube till complete dissolution.
	Standards have been degraded.	Follow suggested storage conditions for standards.
	When pipetting, the required volume is incorrect or inaccurate.	Use calibrated pipette and correct pipetting method.
	Expired kit	Don't use expired products.
	Improper storage	Follow suggested storage conditions for all components.
	The wavelength of the microplate reader is incorrect.	Check the wavelength and read the OD405 or OD414 value again.

High Background	Too many detection reagents or higher concentration.	Use calibrated pipette and correct pipetting method.
	Reading of assay result is not in time.	Read the assay result immediately after test
	The DTNB working solution is contaminated	Reconfigure the working fluid using a clean tube

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.

2. During the Elisa kit development, some endogenous interferons (not all) in the biological sample have been removed or decreased.

3. The final assay result is related to the validity of reagents, experimental operation and environment. Our company is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.

4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.

5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.

6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.

8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

9. This kit allows for research use only. For IVD or other purposes, our company is not responsible for relevant consequences and doesn't bear any legal liability.