Technical Manual

GSH (Reduced Glutathione) colorimetric Assay Kit

- Catalogue Code: AEFI01416
- Colorimetric Assay
- Research Use Only

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1. Key features and Sample Types

Detection method:

Colorimetric

Sample Type:

Serum, Plasma, Cell Culture Supernatant, cell or tissue lysate & other liquid samples

Reactivity:

Universal

Range:

1.56-100umol/L

Sensitivity:

1.2umol/L

Storage:

2-8°C for 6 months (sealed), please do not freeze!

Expiry:

See Kit Label

2. Storage & Expiry

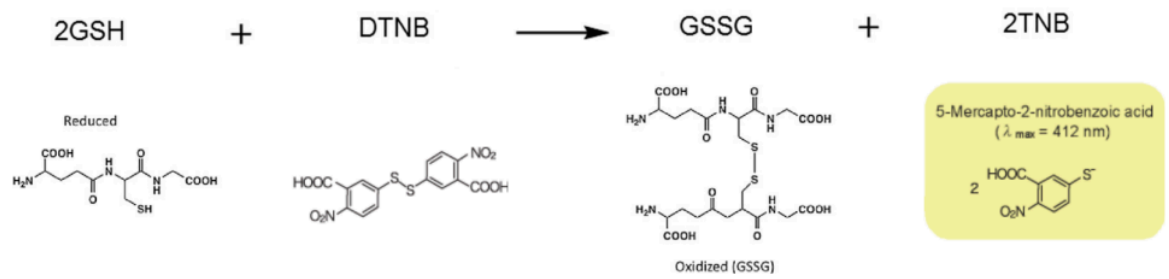
Assay Genie Assays are shipped on ice packs. Please store this unopened Assay Kit at 4°C for 6 months. For opened kits, store individual components as described in the Kit Contents section. Date of expiration will be on the Assay Box label.

3. Description and Principle

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.



How do our ELISA kits work?

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.

4. Kit Contents

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below. Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (Dismountable)	8×6	8×12	Store for 6 months at 2-8°C or
2	GSH Standard (3.07 mg)	1vial	2 vial	
3	Standard stock solution	1.5 mL	3 mL	
4	DTNB (Concentrated, 20X)	270 uL	520 uLl	
5	Sample Dilution Buffer	10ml	20 ml	
6	Assay Dilution Buffer	11ml	22ml	
10	Product Description	1 copy	1 copy	

Additional materials required:

1. Microplate reader (wavelength: 450nm)

2. 37°C incubator (CO2 incubator for cell culture is not recommenced.)

3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)

4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)

5. Sterile tubes and Eppendorf tubes with disposable tips

6. Absorbent paper and loading slot

7. Deionized or distilled water

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plate is kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

10. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

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

5. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolysed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1000 x g. Collect the clear supernatant and assay immediately.

Cell lysates:

- Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes and collect cells. Then add precooling 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 an 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℃ 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.

Tissue Homogenates:

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.

Note: 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 the kit's working.

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

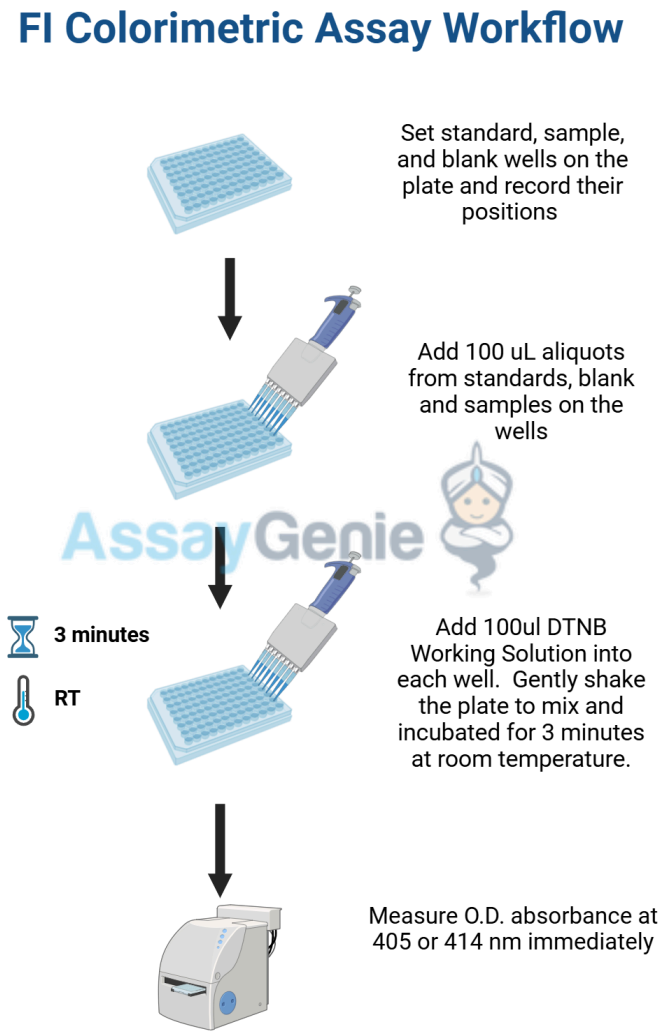
General Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Haemolysed samples are not suitable for this assay.

Notes for Samples

1. Blood Collection Tubes: Use disposable, non-endotoxin blood collection tubes. Avoid using hemolyzed or lipemic samples.
2. Optimal Sample Storage Conditions: Store samples at 2-8°C for up to 5 days. Store at -20°C for up to 6 months. Store at -80°C for up to 2 years. For long-term storage, samples can be stored in liquid nitrogen. When thawing frozen samples, use a rapid water bath (15-25°C) to minimize the formation of ice crystals at 0°C. After thawing, centrifuge the samples to remove any precipitate, then mix well.
3. Detection Range: The detection range of this kit does not directly correspond to the analyte concentration in the sample. For samples with higher or lower concentrations, dilution or concentration may be necessary.
4. Pretest Recommendation: For special samples with no reference data, a pretest is recommended to validate the assay's accuracy and reliability.
5. Recombinant Protein Compatibility**:** Recombinant proteins may not bind effectively to the capture or detection antibodies in the kit, which could result in undetectable assay results.

6. Workflow Overview



7. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350μl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and select the optimal dilution factor to ensure the protein concentration falls within the kit's recommended detection range. Dilute the samples using the dilution buffer provided with the kit. Multiple dilution tests may be necessary to achieve optimal results. Ensure that the test samples are thoroughly mixed with the dilution buffer. Both standard and sample dilutions should be prepared prior to starting the experiment.

Reagent Preparation

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

1. 10mM GSH standard storage solution :

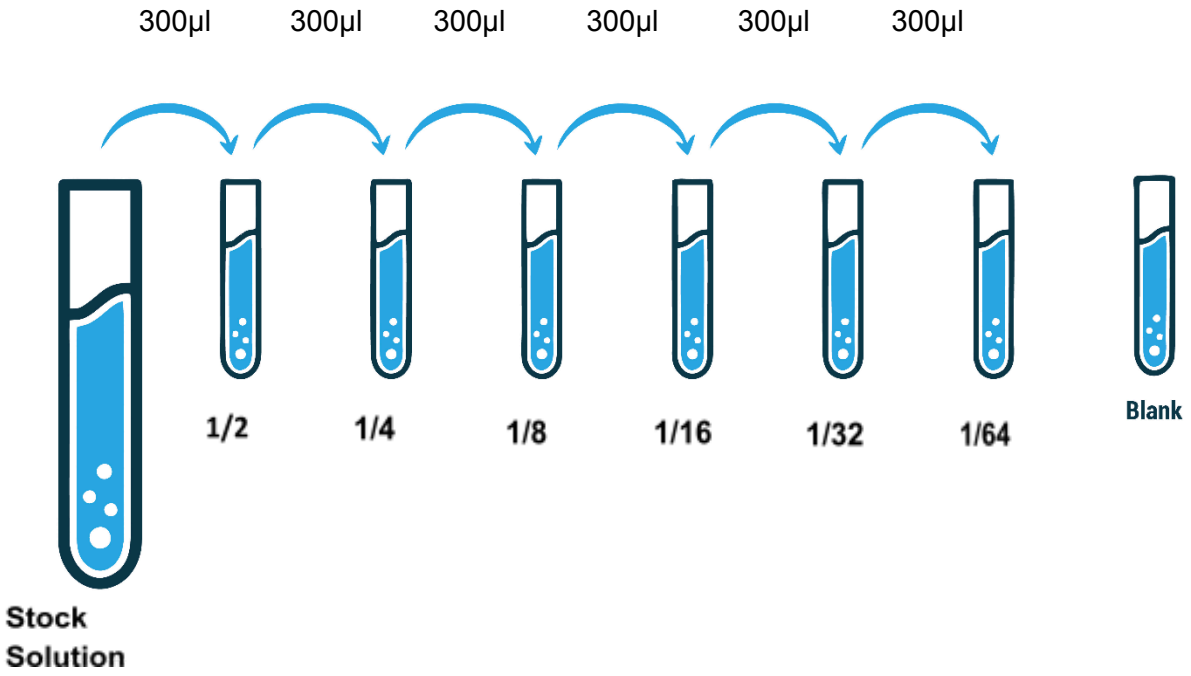
1) Centrifuge GSH standards tube for 1min at 10000xg. Label it as 10mM GSH.
2) Add 1ml Standard stock solution buffer into the standard tube. Tighten the tube cap and invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 20 seconds.)

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

2. Standard Dilution:

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) Centrifuge the tubes for 1min at 1000xg, making the liquid towards the bottom of tube and removing possible bubbles.
3) 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 contain 0.3ml Assay Dilution Buffer.

DILUTION SERIES



Note: 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.

3. Preparation of DTNB Working Solution

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

1) Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)
2) Centrifuge for 10s at 1000xg in low speed and bring down the concentrated DTNB (20X) to the bottom of tube.
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.)

8. 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.

9. Data Analysis

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

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.

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

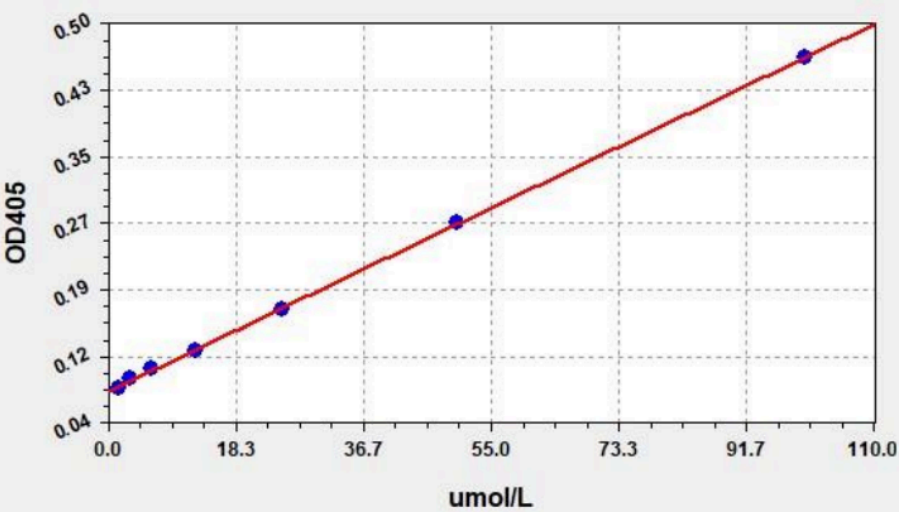
Note：f (sample dilution factor), C (total protein concentration of the samples)

10. Typical Data & Standard Curve

Standard Curve

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)	OD1	OD2	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	0.272
100	0.464	0.462	0.463



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 9n=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 9n=5)	99-105%	89-101%	84-99%

Precision

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

Inter-assay Precision: samples with low, medium and high concentration were 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	56.32

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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