TECHNICAL MANUAL

GSH/GSSG Colorimetric Assay Kit

- **SKU CODE:** MAES0086
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Standard curve preparation
- T-GSH measurement
- GSSG measurement
- Result calculation

2. Intended use

This kit can be used to measure T-GSH and GSSG content in serum (plasma), animal tissue, whole blood, red blood cells and cultured cells samples.

3. Detection principle

GSSG is reduced to GSH by glutathione reductase, and GSH can react with DTNB to produce GSSG and yellow TNB. The amount of total glutathione (GSSG+GSH) determines the amount of yellow TNB. Thus the total glutathione can be calculated by measuring the OD value at 412 nm. The content of GSSG can be determined by first removing GSH from the sample with GSH Scavenger and then applying the aforementioned reaction principle.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	45 mL × 1 vial	-20°C, 12 months
Reagent 2	Standard	6.13 mg × 1 vial	-20°C, 12 months
Reagent 3	Protein Precipitator	50 mL × 2 vials	-20°C, 12 months
Reagent 4	Enzyme Stock Solution	80 µL × 1 vial	-20°C, 12 months
Reagent 5	Chromogenic Agent	1 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 6	GSH Scavenger Auxiliary Solution	1.1 mL × 1 vial	-20°C, 12 months

Item	Component	Size (96 T)	Storage
Reagent 7	GSH Scavenger	0.1 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 8	Substrate	Powder × 1 vial	-20°C, 12 months, protect from light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (405-415 nm, optimum wavelength: 412 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4), Absolute ethanol

6. Reagent preparation

1. Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of 1 mmol/L standard stock solution:** Dissolve one vial of standard with 10 mL of double distilled water, mix well to dissolve. Store aliquoted at -20°C for 1 month.
3. **Preparation of 20 μmol/L Standard solution:** Dilute 20 μL of 1 mmol/L standard stock solution with 980 μL of protein precipitator, mix well. Stable for 24 h when stored at 2-8°C.
4. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the test wells. For example, prepare 540 μL of reaction working solution (mix well 1 μL of enzyme stock solution, 20 μL of chromogenic agent and 519 μL of buffer solution). Stable for 24 h when stored at 2-8°C.
5. **Preparation of GSH scavenger auxiliary working solution:** Before testing, prepare sufficient GSH scavenger auxiliary working solution according to the test wells. For example, prepare 40 μL of GSH scavenger auxiliary working solution

(dilute 20 µL of GSH scavenger auxiliary solution with 20 µL of double distilled water, mix well). Stable for 24 h when stored at 2-8°C.

6. **Preparation of GSH scavenger working solution:** Before testing, prepare sufficient GSH scavenger working solution according to the test wells. For example, prepare 50 µL of GSH scavenger working solution (dilute 5 µL of GSH scavenger with 45 µL of absolute ethanol, mix well). Stable for 24 h when stored at 2-8°C.
7. **Preparation of substrate stock solution:** Dissolve one vial of substrate with 100 µL of double distilled water, mix well to dissolve. Store aliquoted at -70°C for 3 months.
8. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the test wells. For example, prepare 400 µL of substrate working solution (mix well 5 µL of substrate stock solution with 395 µL of buffer solution). Stable for 24 h when stored at 2-8°C.
9. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 20 µmol/L standard solution with protein precipitator to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 5, 8, 10, 15 µmol/L.

7. Sample preparation

Serum and plasma:

1. Prepare serum/plasma using standard methods.

2. Take 100 µL of sample and add 400 µL of protein precipitator, mix thoroughly with a vortex mixer for 30 s, stand for 5 min at 4°C.

3. Centrifuge at 3100×g for 10 min.

4. Take the supernatant and preserve it on ice for detection.

Whole blood:

1. Collect blood, use heparin or EDTA as the anticoagulant.

2. Take 100 µL of whole blood and add 400 µL of protein precipitator, mix thoroughly for 30 s with a vortex mixer, stand for 5 min at 4°C.

3. Centrifuge at 3100×g for 10 min.

4. Take the supernatant and preserve it on ice for detection.

Red blood cell:

1. Collect blood, use heparin or EDTA as the anticoagulant.

2. Centrifuge at 2000 rpm for 10 min immediately, carefully remove the plasma and leukocytic layer (upper layer).

3. Take 100 µL of red blood cells, add 400 µL of protein precipitator, mix thoroughly for 30 s with a vortex mixer, stand for 5 min at 4°C.

4. Centrifuge at 3100×g for 10 min.

5. Take the supernatant and preserve it on ice for detection.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL protein precipitator with a dounce homogenizer at 4°C.

4. Centrifuge at 10000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 200 µL protein precipitator with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Human plasma: 1

10% Rat liver tissue homogenate: 10-20

10% Rat kidney tissue homogenate: 1

10% Rat heart tissue homogenate: 10-20

10% Mouse brain tissue homogenate: 2-5

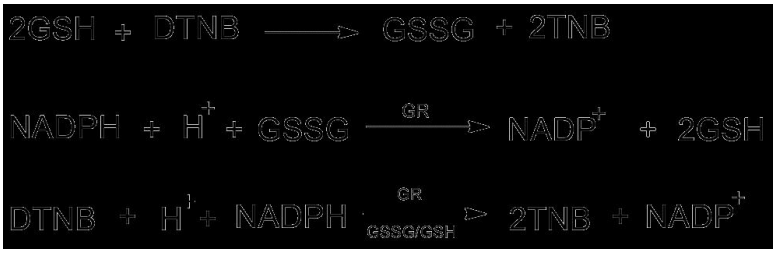
HepG2 cells: 1

Note: The diluent is protein precipitator. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The viscosity of GSH scavenger auxiliary solution is very high, so should be pipetted slowly and carefully.
2. The GSH scavenger has a pungent odor. Please operate in a fume hood.

9. Operating steps



The measurement of T-GSH:

1. Standard well: Take 10 μL of the standard solution with different concentrations to the corresponding wells.

Sample well: Take 10 μL of sample to the corresponding sample wells.

2. Add 150 μL of reaction working solution to each well and incubate at room temperature or 25°C for 5 min.

3. Add 50 μL of substrate working solution to each well, mix thoroughly for 5 s with microplate reader.

4. Incubate at room temperature or 25°C for 25 min and measure the OD value of each well at 412 nm.

The measurement of GSSG:

1. Pretreatment of standard: Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of the standard solution with different concentrations (15, 10, 8, 5, 2, 1, 0.5, 0 μmol/L), mix thoroughly with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix thoroughly with a vortex mixer immediately, react at 25°C for one hour.

2. Remove the GSH of samples: Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of samples (pretreated with protein precipitator in sample preparation step), mix thoroughly with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix thoroughly with a vortex mixer immediately, react at 25°C for one hour.

3. Standard wells of GSSG: Take 10 μL of the standard solution with different concentrations to the corresponding wells.

Sample wells of GSSG: Take 10 μL of sample to the corresponding sample wells.

4. Add 150 µL of reaction working solution to each well and incubate at room temperature or 25°C for 5 min.

5. Add 50 µL of substrate working solution to each well, mix thoroughly for 5 s with microplate reader.

6. Incubate at room temperature or 25°C for 25 min and measure the OD value of each well at 412 nm.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma), whole blood, red blood cells samples:

T-GSH content (µmol/L) = $(\Delta A\_1 - b_1) \div a_1 \times 2^* \times 5^{**} \times f$

GSSG content (µmol/L) = $(\Delta A\_2 - b_2) \div a_2 \times 5^{**} \times f$

2. Tissue sample:

T-GSH content (µmol/g) = $(\Delta A\_1 - b_1) \div a_1 \times 2^* \div m \times V_1 \times f$

GSSG content (µmol/g) = $(\Delta A\_2 - b_2) \div a_2 \div m \times V_1 \times f$

3. Cell sample:

T-GSH content (µmol/10^9) = $(\Delta A\_1 - b_1) \div a_1 \times 2^* \div 1^{***} \times V_2 \times f$

GSSG content (µmol/10^9) = $(\Delta A\_2 - b_2) \div a_2 \div 1^{***} \times V_2 \times f$

Reduced GSH content = T-GSH content - 2×GSSG content

Note:

y: $OD_{Standard}$ - OD_{Blank}

x: The concentration of standard

a_1: The slope of standard curve of T-GSH

b_1: The intercept of standard curve of T-GSH

ΔA_1: OD_{Sample} - OD_{Blank} (for T-GSH)

a_2: The slope of standard curve of GSSG

b_2: The intercept of standard curve of GSSG

ΔA_2: OD_{Sample} - OD_{Blank} (for GSSG)

2*: With GSSG as the standard, need to multiply by 2 when converting to GSH

5**: Dilution multiple of sample in sample preparation step

f: Dilution factor of sample before test

m: the fresh weight of sample

V_1: the volume of protein precipitator in sample preparation step of tissue sample

1***: the cell number, 1×10^6

V_2: the volume of protein precipitator in sample preparation step of cell sample

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	3.50	12.80	22.50
%CV	1.0	0.4	0.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	3.50	12.80	22.50
%CV	4.1	3.6	4.0

Recovery

Three samples of high, middle, and low concentrations were tested 6 times in parallel to obtain an average recovery rate of 97%.

Recovery

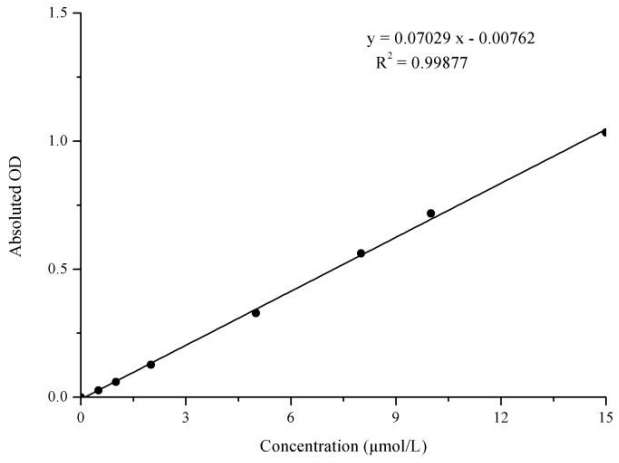
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	0.85	3.5	13.4
Observed Conc. (µmol/L)	0.8	3.4	12.6
Recovery rate (%)	99	98	94

Sensitivity

The analytical sensitivity of the assay is 0.36 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only.

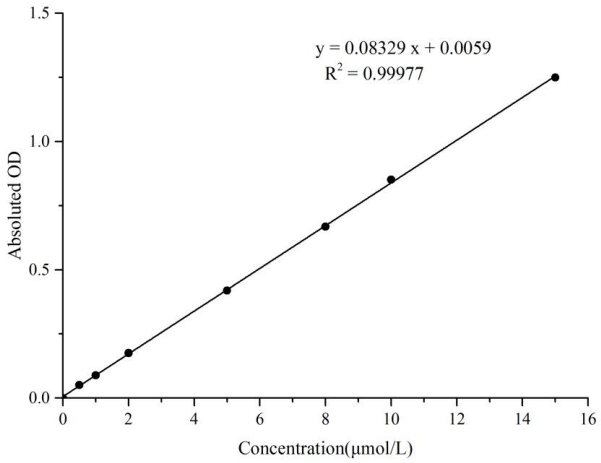


The standard curve of GSSG is as follows:

GSSG Standard Curve Data

Concentration (µmol/L)	Average OD	Absolute OD
0	0.113	0
0.5	0.14	0.027
1	0.173	0.060
2	0.24	0.127
5	0.4415	0.329
8	0.6745	0.562
10	0.831	0.718
15	1.147	1.034

T-GSH Standard Curve Data



The standard curve of T-GSH is as follows:

T-GSH Standard Curve Data

Concentration (µmol/L)	Average OD	Absolute OD
0	0.105	0
0.5	0.156	0.051
1	0.194	0.089

Concentration (μmol/L)	Average OD	Absolute OD
2	0.281	0.176
5	0.524	0.419
8	0.773	0.668
10	0.957	0.852
15	1.355	1.250

12. Appendix II Example Analysis

Example analysis:

Dilute 10% rat liver tissue homogenate with protein precipitator 20 times, then take 10 μL of diluted sample and carry out the assay according to the operation steps. The results are as follows:

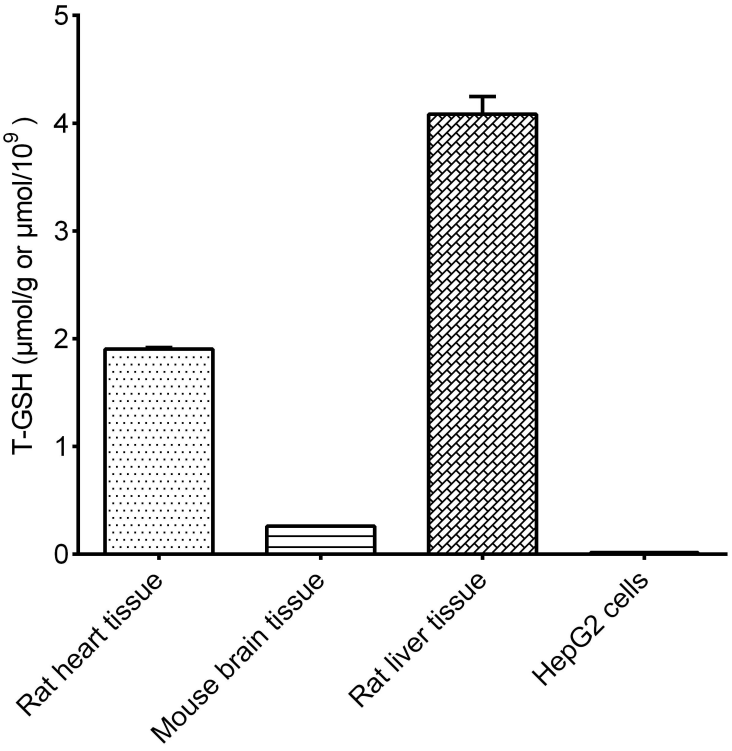
The standard curve of T-GSH: y = 0.0858x + 0.0064, the average OD value of the sample well is 1.159, the average OD value of the blank well is 0.114, the calculation result is:

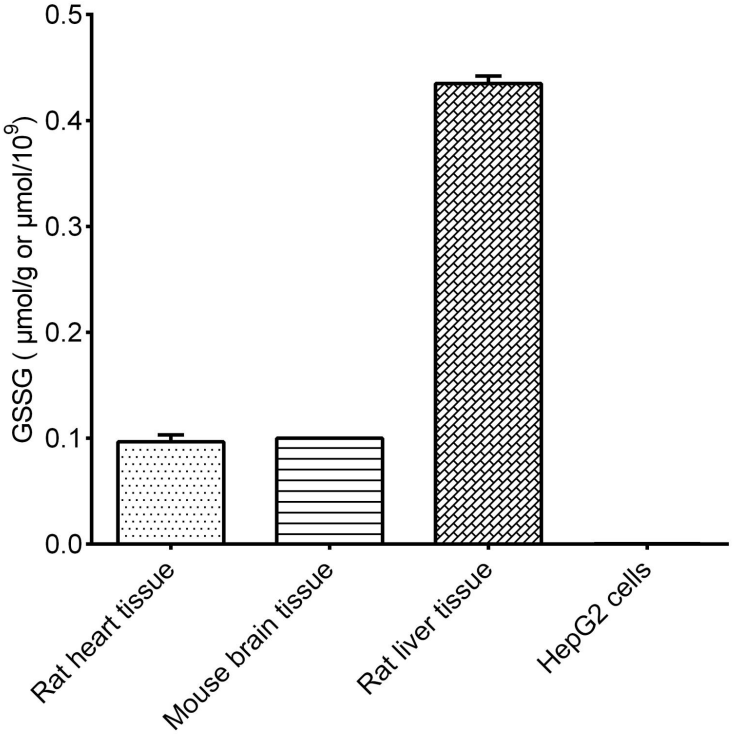
T-GSH (μmol/g) = (1.159 - 0.114 - 0.0064) ÷ 0.0858 × 2 ÷ 0.05 × 0.45 × 10^{-3} × 20 = 4.36 μmol/g

The standard curve of GSSG: y = 0.0717x - 0.007, the average OD value of the sample well is 0.320, the average OD value of the blank well is 0.118, the calculation result is:

GSSG (μmol/g) = (0.320 - 0.118 + 0.007) ÷ 0.0717 ÷ 0.05 × 0.45 × 10^{-3} × 20 = 0.52 μmol/g

Detect 10% rat heart tissue homogenate (diluted 10 times), 10% mouse brain tissue homogenate (diluted 2 times), 10% rat liver tissue homogenate (diluted 20 times), HepG2 cells (diluted 2 times) according to the protocol, the results are as follows:





13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.

3. Protection methods must be taken by wearing lab coat and latex gloves.

4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be performed on the sample.

5. It is recommended to perform a pre-test if your sample is not listed in the instruction manual.

6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Assay Genie will guarantee the quality of the kits only, and will NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.