TECHNICAL MANUAL

Reduced Glutathione (GSH) Colorimetric Assay Kit

- **SKU CODE:** MAES0042
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure absorbance at 405 nm

2. Intended use

This kit can be used to measure the GSH content in serum, plasma, cells, cell culture supernatant and tissue samples.

3. Detection principle

Reduced glutathione (GSH) can react with dinitrobenzoic acid (DNTB) to form a yellow complex which can be detected by colorimetric assay at 405 nm and calculate the reduced GSH content indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	Acid Reagent	6 mL × 1 vial	12 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Phosphate	6 mL × 1 vial	12 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 3	DTNB Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	GSH Standard	3.07 mg × 1 vial	3.07 mg × 2 vials	3.07 mg × 10 vials	2-8°C, 12 months
Reagent 5	GSH Standard Stock Diluent	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (405-414 nm), Micropipettor, Vortex mixer

Reagents:

Distilled or deionized water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **The preparation of GSH standard diluent::** Dilute 1.3 mL of GSH standard stock diluent with 11.7 mL of double-distilled water. The GSH standard diluent should be prepared on the spot.
3. **The preparation of 1 mmol/L GSH standard solution::** Dissolve 3.07 mg of GSH standard with 10 mL of GSH standard diluent. Mix well to dissolve. Aliquot and store at -20°C for 1 month.
4. **The preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L GSH standard solution with GSH standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

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 300-500 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): Human serum (1), Human plasma (1), 10% Mouse brain tissue homogenization (1), 10% Mouse liver tissue homogenization (1), HeLa cell homogenization (0.999 mgprot/mL) (1), Rat serum (1), Rat plasma (1), Mouse serum (1), 10% Carrot tissue homogenization (1), 293T supernatant (1).

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). 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 supernatant after centrifugation must be clarified.
2. Prevent the formation of bubbles when the supernatant is transferred into the microplate.

9. Operating steps

1. Preparation of sample supernatant: take 0.1 mL of sample, add 0.1 mL of acid reagent and mix well. Centrifuge at 4,500×g for 10 min. Collect supernatant for detection.
2. Add 25 µL of DTNB solution to each tube.

3. **Add samples and standards::** Control well: add 100 µL of acid reagent. Standard well: add 100 µL of standard solution with different concentration. Sample well: add 100 µL of supernatant.
4. Add 100 µL of phosphate solution to each tube.
5. Mix well for 5 s and stand for 5 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.

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 by 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) and other liquid sample:

GSH content (µmol/L) = (ΔA_405-b) ÷ a × 2* × f

2. Tissue and cells sample:

GSH content (µmol/gprot) = (ΔA_405-b) ÷ a × 2* × f ÷ C_{pr}

[Note]

ΔA_405: OD_{Sample} – $OD_{Control}$

2*: Dilution factor in the preparation step of sample supernatant, 2 times.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

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).

Inter-assay Precision

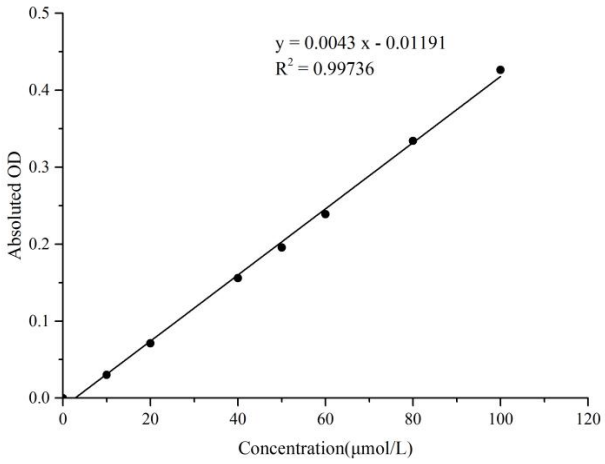
Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 96%.

Sensitivity

The analytical sensitivity of the assay is 2 µmol/L GSH. 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.



12. Appendix II Example Analysis

Example analysis:

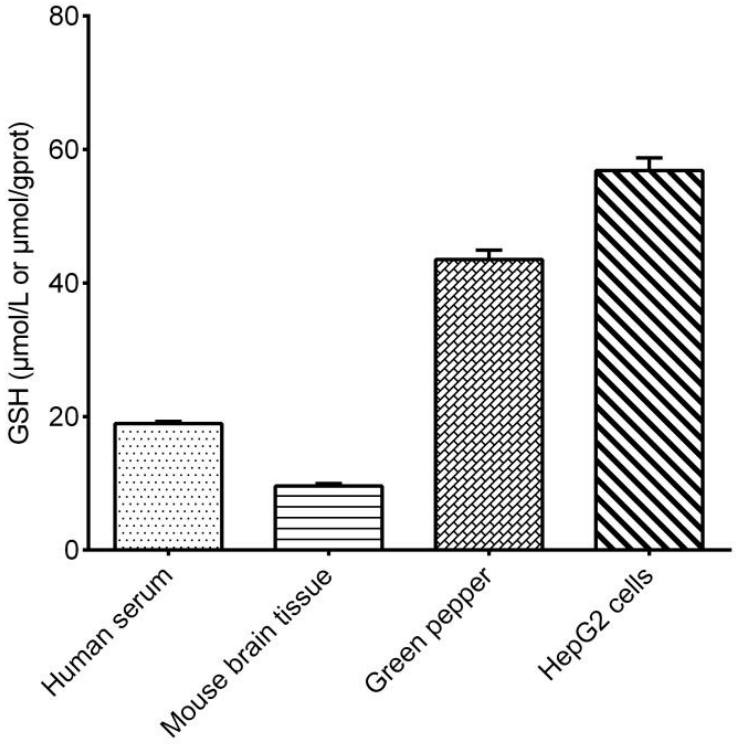
Take 0.1 mL of human serum sample, add 0.1 mL of acid reagent, mix fully and centrifuge at 4,500×g for 10 min, then take prepared supernatant and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.00383x – 0.00251, the average OD value of the sample is 0.080, the average OD value of the control is 0.047, and the calculation result is:

GSH content (µmol/L) = (0.080 - 0.047 + 0.00251) ÷ 0.00383 × 2 = 18.54 µmol/L

Detect human serum, 10% mouse brain tissue homogenate (the concentration of protein is 5.37 gprot/L), 10% green pepper tissue homogenate (the concentration of protein is

1.10 gprot/L), HepG2 cells (the concentration of protein is 3.19 gprot/L) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

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

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.