TECHNICAL MANUAL

Glutathione-S-Transferase (GST) Activity Assay Kit (DTNB)

- **SKU CODE:** MAES0197
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Enzymatic reaction
- Color reaction
- Measure the absorbance at 412 nm

2. Intended use

This kit can be used to measure the GST activity in serum, plasma and animal tissue samples.

3. Detection principle

GST can catalyze the binding of reduced glutathione (GSH) to dinitrobenzene (CDNB). The enzyme activity is determined by measuring the substrate GSH binding rate with dinitrodiphenyl in unit time. The remaining GSH reacts with disulfide double nitro benzoic acid (DTNB) to form yellow glucosinolates nitro benzoic acid anion (TNB), the concentration of which is determined to calculate the reduction of GSH. Thus, the activity of glutathione S-transferase (GST) is calculated indirectly by measuring the OD value at 412 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Substrate	Powder × 1 vial	2-8°C, 12 months
Reagent 2	Stock Diluent	12 mL × 1 vial	2-8°C, 12 months
Reagent 3	Stop Solution	50 mL × 1 vial	2-8°C, 12 months
Reagent 4	Phosphate	15 mL × 1 vial	2-8°C, 12 months
Reagent 5	DTNB Solution	5 mL × 1 vial	2-8°C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 6	Standard	Powder × 1 vial	2-8°C, 12 months
Reagent 7	Standard Stock Solution	3 mL × 1 vial	2-8°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (412 nm), Test tube, Micropipettor, Vortex mixer, Centrifuge, 37°C incubator

Reagents:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 10 mL of stock diluent, mix well to dissolve. Store at 2-8°C for 1 day.
3. **Preparation of standard stock application solution:** Dilute 1.3 mL of standard stock solution with 11.7 mL of double distilled water, mix well. Store at 2-8°C for 3 days.
4. **Preparation of 1 mmol/L standard solution:** Dissolve one vial of standard with 10 mL of standard stock application solution, mix well to dissolve. Store at 2-8°C for 3 days.
5. **Preparation of 250 μmol/L standard solution:** Dilute 300 μL of 1 mmol/L standard solution and 900 μL of standard stock application solution, mix well. Store at 2-8°C for 3 days.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 250 μmol/L GSH standard with standard stock application solution to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 75, 100, 125, 150, 200, 250 μmol/L.

7. Standard curve preparation

Item	1	2	3	4	5	6	7	8
Concentration (μmol/L)	0	25	75	100	125	150	200	250
250 μmol/L GSH standard (μL)	0	30	90	120	150	180	240	300
Standard stock application solution (μL)	300	270	210	180	150	120	60	0

8. 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 PBS (0.01 M, pH 7.4) 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.

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):

Sample type - Dilution factor

Human plasma (serum) - 1

Horse serum - 1

Rat serum - 1

Rabbit serum - 1

Porcine serum - 1

10% Rat kidney tissue homogenate - 1

10% Rat brain tissue homogenate - 1

10% Rat liver tissue homogenate - 1

10% Rat spleen tissue homogenate - 1

10% Rat lung tissue homogenate - 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.

9. The key points of the assay

1. During the color reaction, carefully take the supernatant after the incubation reaction to avoid taking the precipitate.
2. Reaction time and operation time must be strictly controlled.

10. Operating steps

Enzymatic reaction:

1. Control tube: Add 60 µL of substrate working solution to a 1.5 mL EP tube.

Sample tube: Add 60 µL of substrate working solution and 20 µL of sample to a 1.5 mL EP tube.

2. Incubate at 37°C for 30 min.

3. Add 400 µL of stop solution and 20 µL of sample to control tubes, mix fully. Add 400 µL of stop solution to sample tubes and mix fully.

4. Centrifuge at 3500×g for 10 min, take 100 µL of the supernatant for color reaction. (If there is precipitation in the supernatant, transfer the supernatant into a new EP tube and centrifuge again.)

Color reaction:

1. Standard well: Add 100 µL of standard solution with different concentrations into the corresponding wells.

Control well: Add 100 µL of control supernatant into the corresponding wells.

Sample well: Add 100 µL of sample supernatant into the corresponding wells.

2. Add 100 µL of phosphate and 25 µL of DTNB solution into each well.

3. Mix fully for 5 s with microplate reader and stand at room temperature for 5 min. Measure the OD values of each well at 412 nm with microplate reader.

11. 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) sample:

Definition: The enzyme amount of 1 μmol/L of GSH concentration decreased by 1 L of sample per minute at 37°C in the reaction system is defined as 1 unit.

GST activity (U/L) = (ΔA_412 - b) ÷ a ÷ t × 24 × f

2. Tissue sample:

Definition: The enzyme amount of 1 μmol/L of GSH concentration decreased by 1 g of tissue protein per minute at 37°C in the reaction system is defined as 1 unit.

GST activity (U/gprot) = (ΔA_412 - b) ÷ a ÷ t × 24 × f ÷ C_{pr}

[Note]

ΔA_412: $OD_{Control}$ - OD_{Sample}

t: Enzymatic reaction time, 5 min

24: Dilution factor of sample in the enzymatic reaction

C_{pr}: Concentration of protein in sample, mgprot/mL

f: Dilution factor of sample before test

12. 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 (U/L)	5.60	34.80	79.50
%CV	2.1	1.7	1.6

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 (U/L)	5.60	34.80	79.50
%CV	6.0	6.4	6.8

Recovery

Three samples of high concentration, middle concentration and low concentration were tested 6 times in parallel for each concentration to get the average recovery rate of 105%.

Recovery

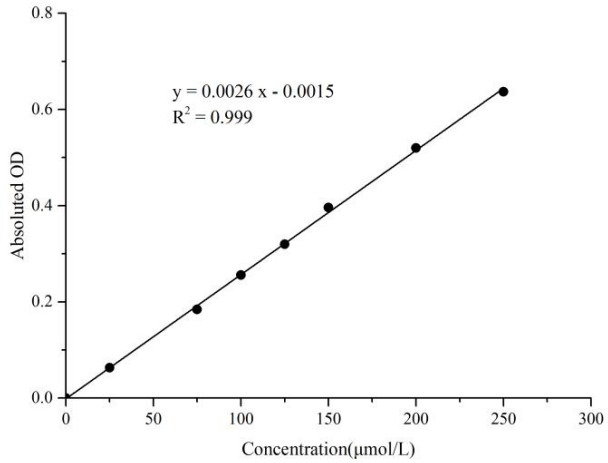
Parameter	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	55	118	180
Observed Conc. (µmol/L)	59.4	122.7	185.4
Recovery rate (%)	108	104	103

Sensitivity

The analytical sensitivity of the assay is 2.1 U/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:



Standard curve

Concentration (μmol/L)	Average OD	Absolute OD
0	0.054	0.000
25	0.117	0.063
75	0.238	0.184
100	0.310	0.256
125	0.374	0.320
150	0.450	0.396
200	0.574	0.520
250	0.691	0.637

13. Appendix II Example Analysis

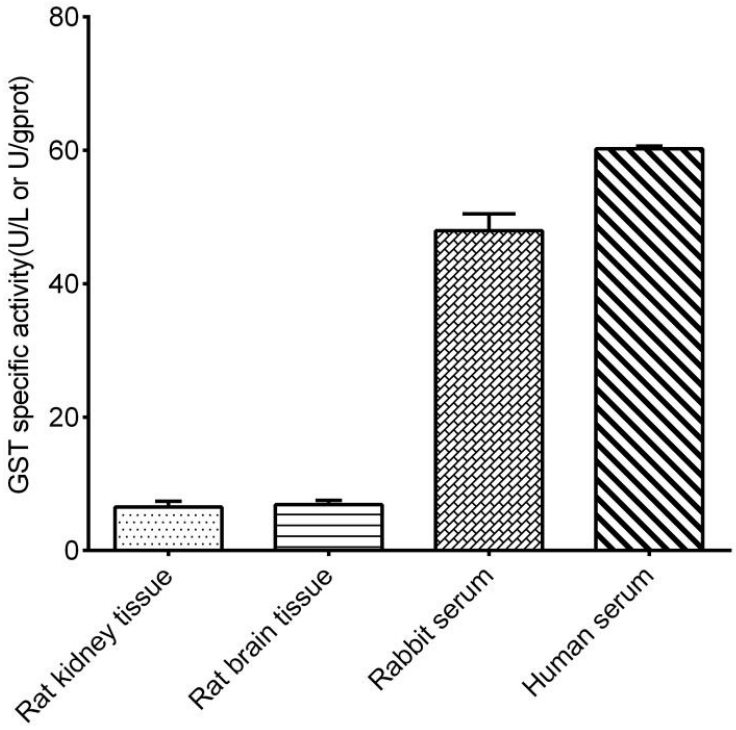
Example analysis:

Take 20 µL of human serum and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0026x – 0.0017, the OD value of the sample is 0.411, the OD value of the control is 0.612, and the calculation result is:

GST activity (U/L) = (0.612 - 0.411 + 0.0017) ÷ 0.0026 ÷ 30 × 24 = 62.4 U/L

Detect 10% rat kidney tissue homogenate (the concentration of protein is 5.36 gprot/L), 10% rat brain tissue homogenate (the concentration of protein is 3.11 gprot/L), rabbit serum and human serum according to the protocol. The result is as follows:



14. 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.