TECHNICAL MANUAL

Glutathione-S-Transferase (GST) Activity Assay Kit

- **SKU CODE:** MAES0169
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Operating steps
- Calculation

2. Intended use

This kit can be used to measure the Glutathione-S-Transferase (GST) activity in serum, plasma, tissue and cell samples.

3. Detection principle

GST can catalyze the binding of reduced glutathione (GSH) to dinitrobenzene (CDNB) and the product has an absorption peak at 340 nm. The activity of GST can be calculated by measuring the increasing rate of absorbance at 340 nm.

4. Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 3	Powder	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months

5. Materials prepared by users

Instruments: Spectrophotometer (340 nm), Micropipettor, Incubator, Vortex mixer

Reagents: Double distilled water

Note: The reagents must be stored strictly according to the preservation conditions in the table above. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to obtain sufficient reagent amounts.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of powder application solution:** Dilute one vial of powder with 5 mL of double distilled water, mix well. Store at 2-8°C for 3 days.

7. Sample preparation

1. **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 extracting solution with a dounce homogenizer 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. 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 μL extracting solution 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.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): Human serum (plasma): 1 10% Rat liver tissue homogenate: 150-200 10% Rat lung tissue homogenate: 8-12 10% Rat kidney tissue homogenate: 10-15 10% Plant tissue homogenate: 1 Note: The diluent is double distilled water or extracting solution. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. Operating steps

1. Preheat the needed powder application solution, buffer solution and cuvette at 37°C for 10 min. Set the spectrophotometer to 340 nm and zero the spectrophotometer with double distilled water.
2. Blank tube: add 0.1 mL of extracting solution to a 2 mL EP tube. Sample tube: add 0.1 mL of sample to a 2 mL EP tube.
3. Add 0.9 mL of buffer solution and 0.1 mL of powder application solution to each tube, mix fully and record the time immediately. Measure the absorbance at 340 nm at 20 s (A_1), incubate at 37°C and measure the absorbance at 340 nm at 320 s (A_2). Calculate the ΔA_blank or sample = A_2 - A_1.

9. Calculation

1. **Serum (plasma) and other liquid samples:** Definition: The amount of GST in 1 mL of sample that catalyzes the combination of 1 μmol of CDNB and GSH at 37°C per minute is defined as 1 unit. GST activity (U/mL) = ΔA / (ε × d × 10^6 ÷ t × V_1 / V_2) × f
2. **Tissue and cell samples:** Definition: The amount of GST in 1 mg of tissue protein that catalyzes the combination of 1 μmol of CDNB and GSH at 37°C per minute is defined as 1 unit. GST activity (U/mgprot) = ΔA / (ε × d × 10^6 ÷ t × V_1 / V_2) × f ÷ C_pr

10. Notes

ΔA: ΔA_Sample – ΔA_Blank

ε: molar extinction coefficient of the product, 9.6×10^3 L/mol/cm

d: optical path of the cuvette, 1 cm

10^6: 1 mol = 10^6 μmol

V_1: the total volume of the reaction system, (1.1 mL = 0.0011 L)

V_2: the volume of sample added into the reaction system, 0.1 mL

t: reaction time, 5 min

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

f: dilution factor of sample before test

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 Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.60	24.50	52.30
%CV	2.3	1.8	1.6

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.60	24.50	52.30
%CV	4.2	4.3	4.4

Recovery

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

Recovery Results

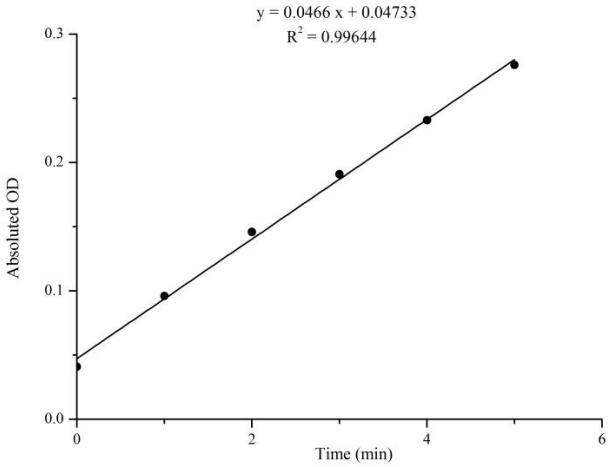
Sample	Expected Conc. (U/L)	Observed Conc. (U/L)	Recovery rate (%)
Sample 1	12.6	13.0	103
Sample 2	32.5	33.8	104
Sample 3	65	70.2	108

Sensitivity

The analytical sensitivity of the assay is 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 data

Time (min)	Absolute OD
0	0.041
1	0.096
2	0.146
3	0.191
4	0.233
5	0.276

12. Appendix II Example Analysis

Example analysis:

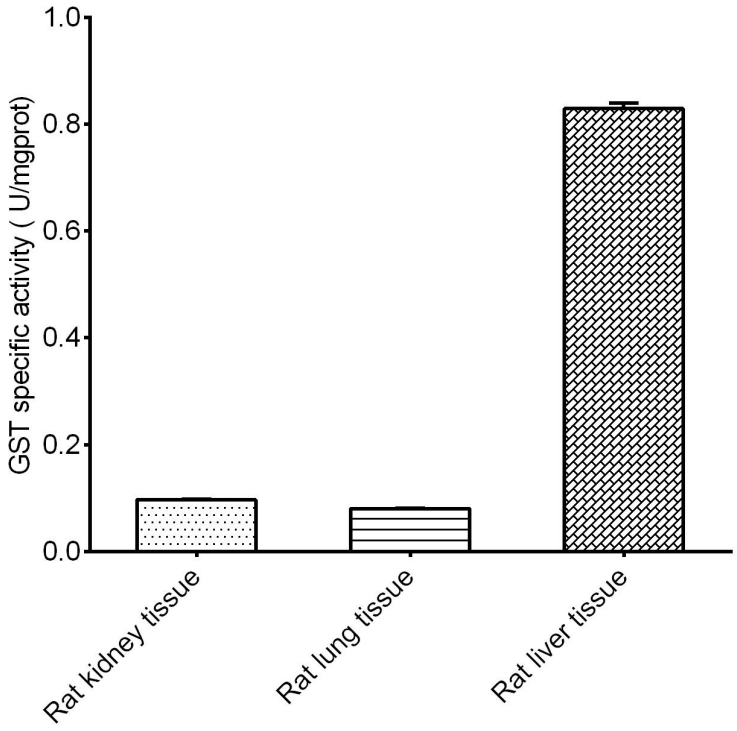
Dilute 10% rat kidney tissue homogenate with extracting solution at a ratio of 1:14, take 0.1 mL of diluted sample and perform the assay according to the operation steps. The results are as follows:

The average OD value of the sample (A_1) is 0.464, the average OD value of the sample (A_2) is 0.729, the average OD value of the blank (A_1) is 0.414, the average OD value of the blank (A_2) is 0.437, the concentration of protein in sample is 8.51 mgprot/mL and the calculation result is:

$\Delta A = (0.729 - 0.464) - (0.437 - 0.414) = 0.242$

GST activity (U/mgprot) = 0.242 / (1×9.6×10^3 × 10^6 ÷ 5 × 0.0011 / 0.1) × 15 ÷ 8.51 = 0.098 U/mgprot

Detect 10% rat kidney tissue homogenate (the concentration of protein is 8.51 mgprot/mL, diluted 15 times), 10% rat lung tissue homogenate (the concentration of protein is 4.72 mgprot/mL, diluted 8 times), 10% rat liver tissue homogenate (the concentration of protein is 13.74 mgprot/mL, diluted 200 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 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. The 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.