CO-K278-S Glutathione-S-Transferase (GSH-ST) Activity Assay Kit

Method: Colorimetric method
Specification: 100 Assays **(**Can detect 96 samples without duplication)
Instrument: Spectrophotometer
Sensitivity: 1 U/L
Detection range: 1-79 U/L

1.1 Intended use

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

1.2 Background

According to the localization of cell, Glutathione-S-transferase (GSH-ST) is divided into three subfamilies: cytoplasmic GSH-ST, microsomal GSH-ST and peroxisome/mitochondrial GSH-ST. GSH-ST is a kind of detoxifying enzyme. Maintain cell integrity by catalyzing the binding of reduced glutathione to electron-friendly substance.

1.3 Detection principle

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

1.4 Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	2-8℃, 12 months
Reagent 2	Buffer Solution	50 mL × 1 vial	2-8℃, 12 months
Reagent 3	Powder	1 vial	2-8℃, 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

1.5 Materials prepared by users

Instruments:
Spectrophotometer (340 nm), Micropipettor, Incubator, Vortex mixer
Consumptive material:
Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)
Reagents:
Double distilled water,

1.6 Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

1.7 Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

1.8 The key points of the assay

1. The reagent 3 application solution and cuvette should be preheat at 37℃ for 10 min
2. The reaction time must be accurate.

2. Pre-assay preparation

2.1 Reagent preparation

Reagent 3 application solution: Dissolve a vial of powder with 5 mL double distilled water and mix fully. Prepared solution can be stored at 2-8℃ for 3 days.

2.2 Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

2.3 Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and the results of the samples are linear within 5 min.
The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
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 reagent 1.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	340 nm

Instructions for the use of transferpettor:
(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.
(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

3. Assay protocol

3.1 Operating steps

1. Preheat the needed reagent 3 application solution and cuvette at 37℃ for 10 min. Set the spectrophotometer to 340 nm and set the spectrophotometer to zero with double distilled water.
2. **Blank well:** add 0.1 mL of reagent 1 to a 2 mL EP tube.
   Sample well: add 0.1 mL of sample to a 2 mL EP tube.
3. Add 0.9 mL of reagent 2 and 0.1mL of reagent 3 application solution to each tube, mix fully and record the time immediately. Measure the absorbance at 340 nm at 20 s (A1) and 320 s (A2), respectively. Calculate the $\triangle A_{\text{blank or sample}}$ =A2-A1.

3.2 Operation table

Preheat the Reagent 3 application solution and cuvette at 37℃ for 10 min.		
	Blank tube	Sample tube
Reagent 1 (mL)	0.1	
Sample (mL)		0.1
Reagent 2 (mL)	0.9	0.9
Reagent 3 application solution (mL)	0.1	0.1
Mix fully and record the time immediately. Measure the absorbance at 340 nm at 20 s (A_1) and 320 s (A_2), respectively. Calculate the $\triangle A_{blank\ or\ sample}=A_2-A_1$.		

3.3 Calculation

1. **Serum (plasma) and other liquid sample:**

   Definition: The amount of GSH-ST in 1 mL of sample that catalyze the combination of 1 μmol of CDNB and GSH at 37℃ per minute is defined as 1 unit.

$$\text{GST activity (U/mL)} = \frac{\Delta A}{\varepsilon \times d} \times 10^6 \div t \times \frac{V_1}{V_2} \times f$$

2. **Tissue and cells sample:**

   Definition: The amount of GSH-ST in 1 mg of tissue protein that catalyze the combination of 1 μmol of CDNB and GSH at 37℃ per minute is defined as 1 unit.

$$\text{GST activity (U/mgprot)} = \frac{\Delta A}{\varepsilon \times d} \times 10^6 \div t \times \frac{V_1}{V_2} \times f \div C_{pr}$$

[Note]

ΔA: $\Delta A_{Sample} - \Delta A_{Blank}$.

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;

Cpr: concentration of protein in sample, mgprot/mL;

f: dilution factor of sample before test.

3.4 Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.

4. Appendix I Performance characteristics

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Detection range	1-79 U/L	**Average intra-assay CV (%)**	1.9
Sensitivity	1 U/L	**Average inter-assay CV (%)**	4.3
Average recovery rate (%)	105		

4.1 Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV ($\bar{R}$) is 4.3%.

$$\bar{x}_T = \frac{\bar{x}_1 + \bar{x}_2 + \bar{x}_3}{3}$$

$$R = \frac{\bar{x}_{max} - \bar{x}_{min}}{\bar{x}_T} \times 100\%$$

$$\bar{R} = \frac{R_1+R_2+R_3}{3} \quad (n=3)$$

$\bar{x}_{max}$--- The max values of $\bar{x}_i$

$\bar{x}_{min}$--- The min values of $\bar{x}_i$

$\bar{x}_T$---The average values of $\bar{x}_i$

R_i---The value of each batch number kit

4.2 Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 1.9%, which was calculated according to the following formula.

$$CV = \frac{s}{\bar{x}} \times 100\%$$

s--- Standard deviation

4.3 Sensitivity

The volume of sample added to reaction is 0.1 mL, carry the assay according to the operation table, the minimum detected absorbance is 0.005, calculate the sensitivity according to the formula in calculation is 1 U/L.

4.4 Recovery rate

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

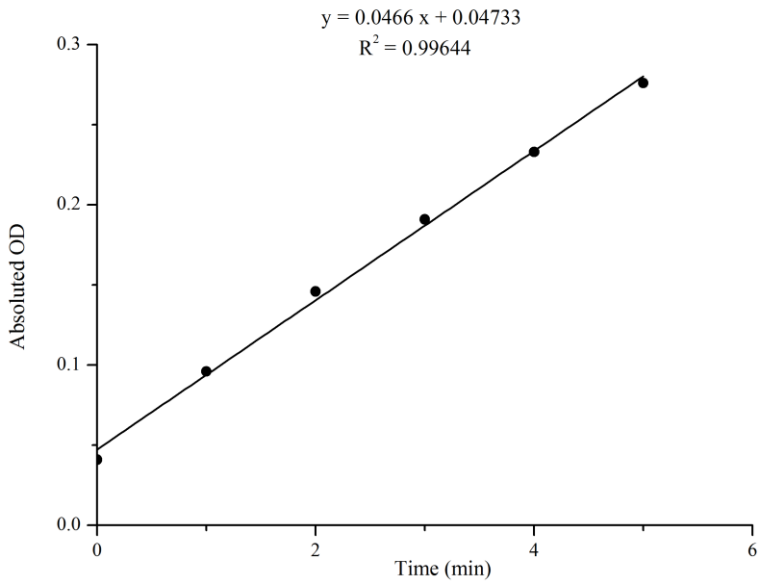
4.5 Response curve of sample

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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

Take 10% rat liver tissue, dilute the sample for 200 times with reagent 1, take 0.1 mL of diluted sample and carry the assay according to the operation table.

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



4.6 Example analysis

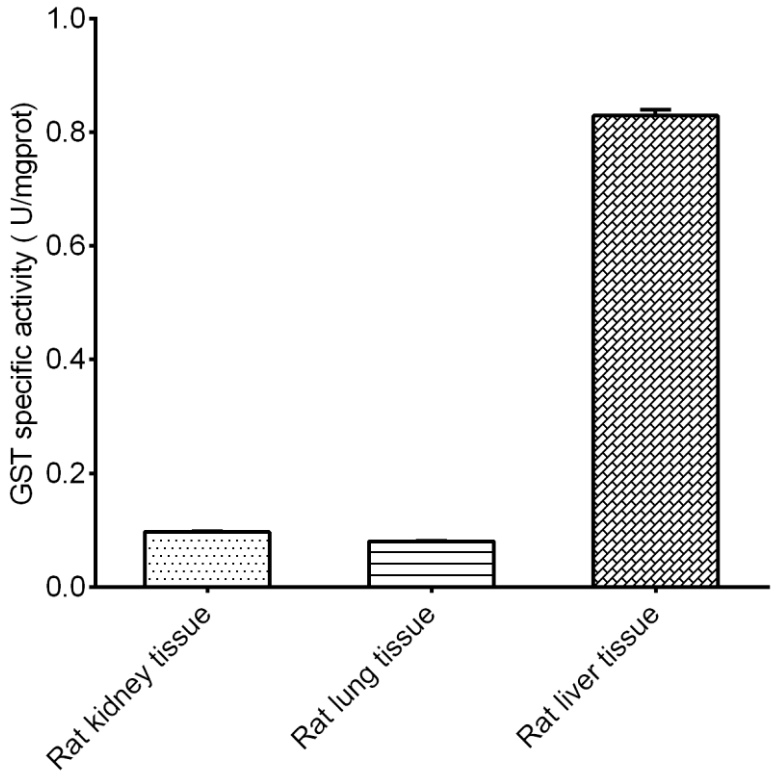
Dilute 10% rat kidney tissue homogenate with reagent 1 at the ratio of 1:14, take 0.1 mL of diluted sample and carry the assay according to the operation table. The results are as follows:

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:

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

$$\text{GST activity（U/mgprot）} = \frac{0.242}{1\times 9.6\times 10^3}\times 10^6 \div 5 \times \frac{0.0011\ \text{L}}{0.1\ \text{mL}} \times 15 \div 8.51\ \text{mgprot/mL} = 0.098\ \text{U/mgprot}$$

Detect 10% rat kidney tissue homogenate (the concentration of protein is 8.51 mgprot/mL, dilute for 15 times), 10% rat lung tissue homogenate (the concentration of protein is 4.72 mgprot/mL, dilute for 8 times), 10% rat liver tissue homogenate (the concentration of protein is 13.74 mgprot/mL, dilute for 200 times) according to the protocol, the result is as follows:



5. Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

5.1 Serum:

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

5.2 Plasma:

Take fresh blood into the tube which has anticoagulant (Heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

5.3 Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the

weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

5.4 Cells:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (106): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Reagent 1.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.

6. Appendix III References

1. Dasari S, Gonuguntla S, Ganjayi M S, et al. Genetic polymorphism of glutathione S-transferases: Relevance to neurological disorders.[J]. Pathophysiology, 2018.

2. Remmerie B, Vandenbroucke K, Smet L D, et al. Expression, purification, crystallization and structure determination of two glutathione S-transferase-like proteins from Shewanella oneidensis[J].Acta Crystallographica, 2008, 64(6): 548-553.