Product Information & ELISA Manual

Glutathione S-Transferase/GST Activity Assay Kit (Colorimetric)

NBP3-24500

Enzyme-linked Immunosorbent Assay
for quantitative detection.

Contact

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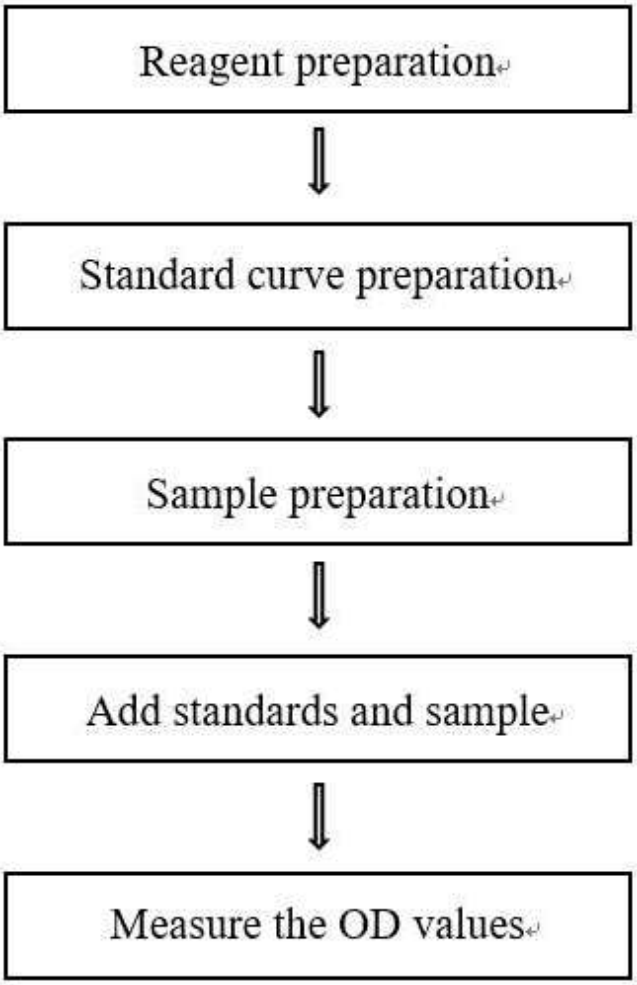
Novus kits are guaranteed for 6 months from date of receipt.

For research use only. Not for diagnostic or therapeutic procedures.

Table of contents

Assay summary



Intended use

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

Detection principle

GST can catalyze the binding of reduced glutathione (GSH) to dinitrobenzene (CDNB). The enzyme activity is indicated by measuring the substrate GSH binding rate with dinitrodiphenyl in unit time, the reaction of the rest of the GSH acts 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) was calculated indirectly by measuring the OD value at 412 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Substrate	Powder × 1 vial	2-8℃, 12 months
Reagent 2	Stock Diluent	12 mL × 1 vial	2-8℃, 12 months
Reagent 3	Stop Solution	50 mL × 1 vial	2-8℃, 12 months
Reagent 4	Phosphate	15 mL × 1 vial	2-8℃, 12 months
Reagent 5	DTNB Solution	5 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	2-8℃, 12 months
Reagent 7	Standard Stock Solution	3 mL × 1 vial	2-8℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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.

For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Equilibrate all reagents to room temperature before use..

② The preparation of substrate working solution：
Dissolve one vial of substrate with 10 mL of stock diluent, mix well to dissolve. Store at 2-8 ℃ for 1 day.

③ The 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 ℃ for 3 days.

④ The 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 at2-8 ℃ for 3 days.

⑤ The 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 ℃ for 3 days.

⑥ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions fter 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. Reference is as follows:

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

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℃ 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℃.

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

⑤ Meanwhile, determine the protein concentration of supernatant (NBP3-25873).

② 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 do pretest to confirm the dilution factor

The key points of the assay

① During the color reaction, take the supernatant carefully after the incubation reaction to avoid take the precipitate.

② Reaction time and operation time must be strictly controlled.

Operating steps

Enzymatic reaction

① Control tube: take 60 μL of substrate working solution to a 1.5 mL EP tube.
Sample tube: take 60 μL of substrate working solution and 20 μL of sample to a 1.5 mL EP tube.

② Incubate at 37℃ for 30 min.

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

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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

$$\text{GST activity (U/L)} = (\Delta A_{412} - b) \div a \div t \times 24 \times f$$

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.

$$\text{GST activity (U/gprot)} = (\Delta A_{412} - b) \div a \div t \times 24 \times f \div C_{pr}$$

[Note]

ΔA_{412}: $OD_{Control} - OD_{Sample}$.

t: Enzymatic reaction time, 5 min.

24: Dilution factor of sample in the enzymatic reaction.

Cpr: Concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.60	34.80	79.50
%CV	6.0	6.4	6.8

Recovery

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%.

	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

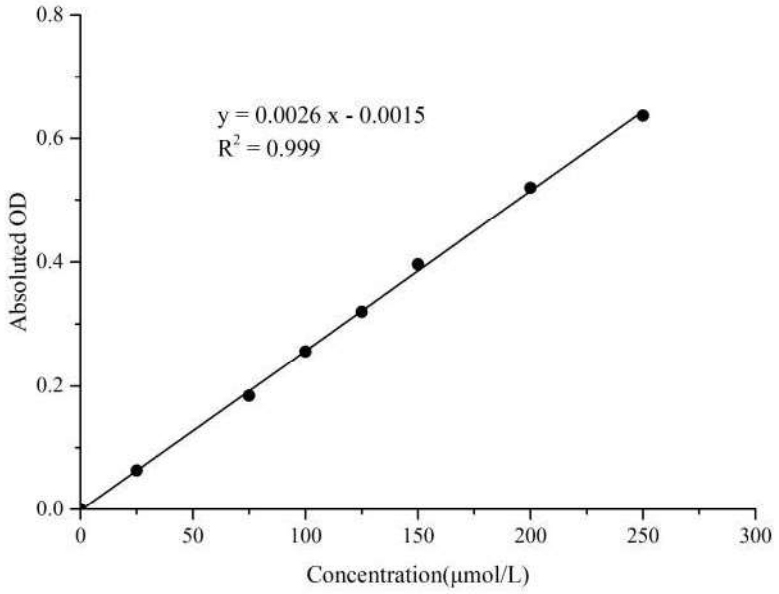
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	1.0	2.0	4.0	5.0	6.0	7.0	8.0
Average OD	0.054	0.117	0.238	0.310	0.374	0.450	0.574	0.691
Absoluted OD	0.000	0.063	0.184	0.256	0.320	0.396	0.520	0.637



Appendix Π Example Analysis

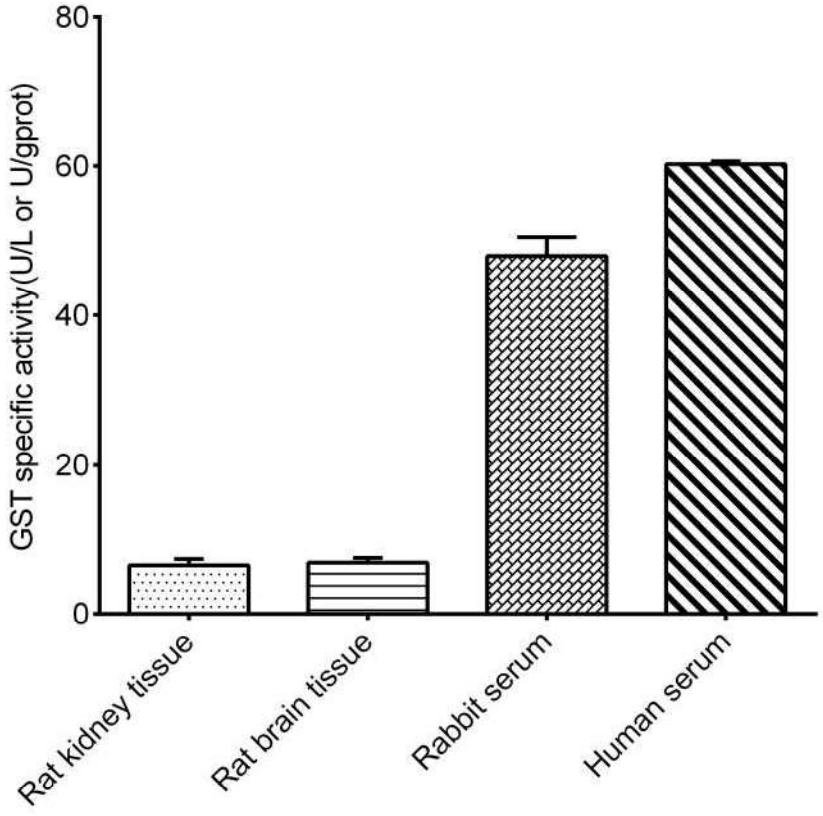
Example analysis:

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

Standard curve: y = 0.0026 x – 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:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
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 taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Novus Biologicals will guarantee the quality of the kits only, and 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.