Product Information & ELISA Manual

Total Glutathione (T-GSH)/Oxidized Glutathione(GSSG) AssayKit(Colorimetric)

NBP3-25936

Enzyme-linked Immunosorbent Assay
for quantitative detection.

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

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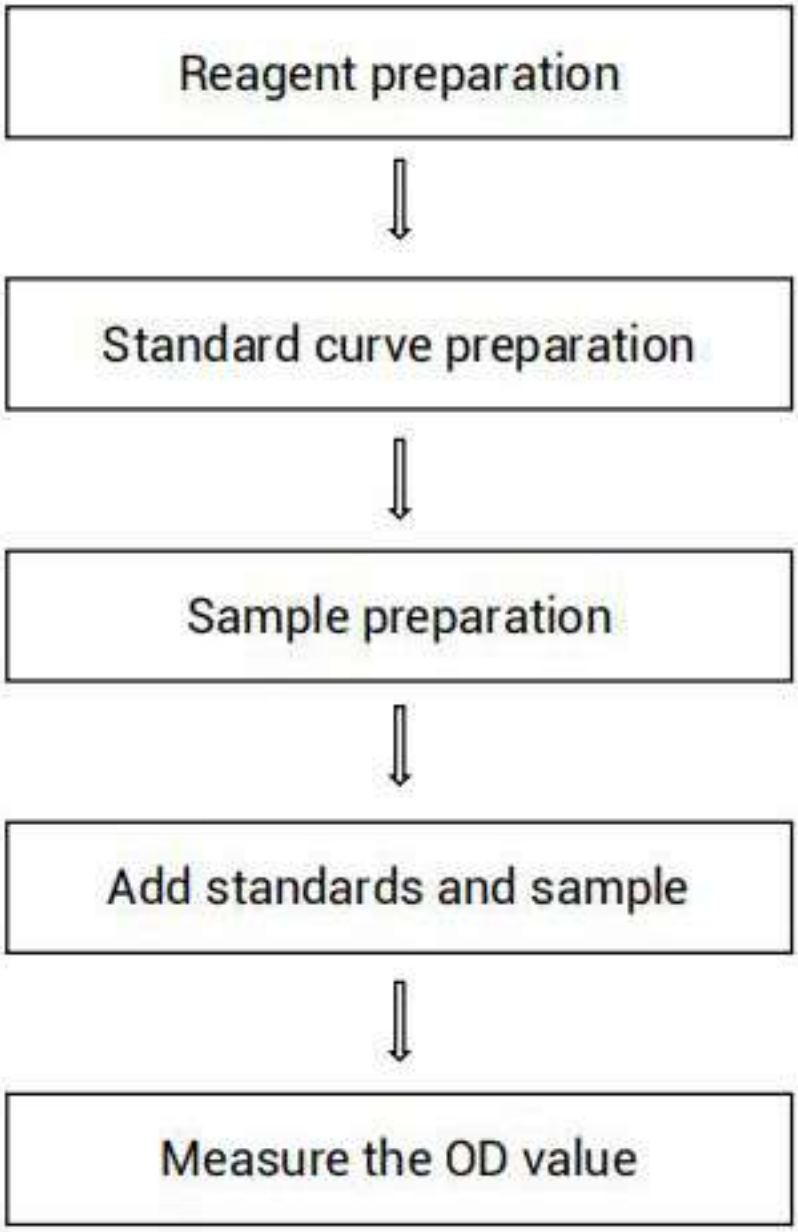
Assay summary

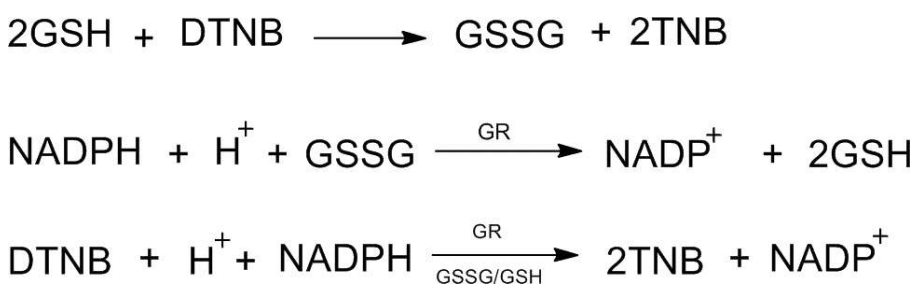


Intended use

This kit can be used to measure T-GSH and GSSG content in serum (plasma), animal tissue, whole blood, red blood cells and cultured cells samples.

Detection principle

GSSG is reduced to GSH by glutathione reductase, and GSH can react with DTNB to produce GSSG and yellow TNB. The amount of total glutathione (GSSG+GSH) determines the amount of yellow TNB. Thus the total glutathione can be calculated by measuring the OD value at 412 nm. The content of GSSG can be determined by first removing GSH from the sample with GSH Scavenger and then applying the aforementioned reaction principle. The Detection range is specified as : 0.36-30 μmol/L T-GSH.



Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	45 mL × 1 vial	-20℃, 12 months
Reagent 2	Standard	6.13 mg × 1 vial	-20℃, 12 months
Reagent 3	Protein Precipitator	50 mL × 2 vials	-20℃, 12 months
Reagent 4	Enzyme Stock Solution	80 μL × 1 vial	-20℃, 12 months
Reagent 5	Chromogenic Agent	1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	GSH Scavenger Auxiliary Solution	1.1 mL × 1 vial	-20℃, 12 months
Reagent 7	GSH Scavenger	0.1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 8	Substrate	Powder × 1 vial	-20℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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 (405-415 nm, optimum wavelength: 412 nm),

Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

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

Reagent preparation

① Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of 1 mmol/L standard stock solution：
Dissolve one vial of standard with 10 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 1 month.

③ The preparation of 20 μmol/L Standard solution：
Dilute 20 μL of 1 mmol/L standard stock solution with 980 μL of protein precipitator, mix well. Stable for 24 h when stored at 2-8°C.

④ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 540 μL of reaction working solution (mix well 1 μL of enzyme stock solution, 20 μL of chromogenic agent and 519 μL of buffer solution). Stable for 24 h when stored at 2-8°C.

⑤ The preparation of GSH scavenger auxiliary working solution：
Before testing, please prepare sufficient GSH scavenger auxiliary working solution according to the test wells. For example, prepare 40 μL of GSH scavenger auxiliary working solution (dilute 20 μL of GSH scavenger auxiliary solution with 20 μL of double distilled water, mix well. Stable for 24 h when stored at 2-8°C.

⑥ The preparation of GSH scavenger working solution：
Before testing, please prepare sufficient GSH scavenger working solution according to the test wells. For example, prepare 50 μL of GSH scavenger working solution (dilute 5 μL of GSH scavenger with 45 μL of absolute ethanol, mix well. Stable for 24 h when stored at 2-8°C.

⑦ The preparation of substrate stock solution：
Dissolve one vial of substrate with 100 μL of double distilled water,

mix well to dissolve. Aliquoted storage at -70°C for 3 months.

⑧ The preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 400 μL of substrate working solution (mix well 5 μL of substrate stock solution with 395 μL of buffer solution, mix well. Stable for 24 h when stored at 2-8°C.

⑨ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 20 μmol/L standard solution with protein precipitator to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 5, 8, 10, 15 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**5**	**8**	**10**	**15**
20 μmol/L standard (μL)	0	10	20	40	100	160	200	300
Protein precipitator (μL)	400	390	380	360	300	240	200	100

Sample preparation

① Sample preparation

Serum and plasma:

① Prepare serum/plasma as the common method.

② Take 100 μL of sample and add 400 μL of protein precipitator, mix fully by a vortex mixer for 30 s, stand for 5 min at 4℃.

③ Centrifuge at 3100×g for 10 min

④ Take the supernatant and preserve it on ice for detection.

Whole blood:

① Collect blood, use heparin or EDTA as the anticoagulation.

② Take 100 μL of whole blood and add 400 μL of protein precipitator, mix fully for 30 s with a vortex mixer, stand for 5 min at 4℃.

③ Centrifuge at 3100×g for 10 min.

④ Take the supernatant and preserve it on ice for detection.

Red blood cell:

① Collect blood, use heparin or EDTA as the anticoagulation.

② Centrifuge at 2000 rpm for 10 min immediately, remove the plasma and leukocytic layer (upper layer) carefully.

③ Take 100 μL of red blood cell, add 400 μL of protein precipitator, mix fully for 30 s with a vortex mixer, stand for 5 min at 4℃.

④ Centrifuge at 3100×g for 10 min.

⑤ Take the supernatant and preserve it on ice for detection.

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 protein precipitator 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.

Cell (adherent or suspension) samples:

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 200 μL protein precipitator with a ultrasonic cell disruptor 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.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human plasma	1
10% Rat liver tissue homogenate	10-20
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	10-20
10% Mouse brain tissue homogenate	2-5
HepG2 cells	1

Note: The diluent is protein precipitator. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The viscosity of GSH scavenger auxiliary solution is very high, so should be pipetted slowly slowly and carefully.

② The GSH scavenger has a pungent odor. Please operate in the fume hood.

Operating steps

The measurement of T-GSH

① Standard well: take 10 μL of the standard solution with different concentration to the corresponding wells.
Sample well: take 10 μL of sample to the corresponding sample wells.

② Add 150 μL of reaction working solution to each well and incubate at room temperature or 25℃ for 5 min.

③ Add 50 μL of substrate working solution to each well, mix fully for 5 s with microplate reader.

④ Incubate at room temperature or 25℃ for 25 min and measure the OD value of each well at 412 nm.

The measurement of GSSG

① The pretreatment of standard: Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of the standard solution with different concentration (15, 10, 8, 5, 2, 1, 0.5, 0 μmol/L), mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix fully with a vortex mixer immediately, react at 25℃ for an hour.

② Remove the GSH of samples: Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of samples (pretreated with protein precipitator in sample preparation step) , mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix fully with a vortex mixer immediately, react at 25℃ for an hour.

③ Standard wells of GSSG: take 10 μL of the standard solution with different concentration to the corresponding wells.
Sample wells of GSSG: take 10 μL of sample to the corresponding sample wells.

④ Add 150 μL of reaction working solution to each well and incubate at room temperature or 25℃ for 5 min.

⑤ Add 50 μL of substrate working solution to each well, mix fully for 5 s with microplate reader.

⑥ Incubate at room temperature or 25℃ for 25 min and measure the OD value of each well at 412 nm.

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), whole blood, red blood cells samples:

$$\underset{(\mu mol/L)}{T-GSH\ content} = (\Delta A_1 - b_1) \div a_1 \times 2* \times 5** \times f$$

$$\underset{(\mu mol/L)}{GSSG\ content} = (\Delta A_2 - b_2) \div a_2 \times 5** \times f$$

2. Tissue sample:

$$\underset{(\mu mol/g)}{T\text{-}GSH\ content} = (\Delta A_1 - b_1) \div a_1 \times 2* \div \frac{m}{V_1} \times f$$

$$\underset{(\mu mol/g)}{GSSG\ content} = (\Delta A_2 - b_2) \div a_2 \div \frac{m}{V_1} \times f$$

3. Cell sample:

$$\underset{(\mu mol/10^9)}{T\text{-}GSH\ content} = (\Delta A_1 - b_1) \div a_1 \times 2* \div \frac{1***}{V_2} \times f$$

$$\underset{(\mu mol/10^9)}{GSSG\ content} = (\Delta A_2 - b_2) \div a_2 \div \frac{1***}{V_2} \times f$$

Reduced GSH content = T-GSH content - 2×GSSG content

[Note]

y: $OD_{Standard} - OD_{Blank.}$

x: The concentration of standard.

a_1: The slope of standard curve of T-GSH.

b_1: The intercept of standard curve of T-GSH.

ΔA_1: $OD_{Sample} - OD_{Blank}$ (for T-GSH)

a_2: The slope of standard curve of GSSG.

b_2: The intercept of standard curve of GSSG.

ΔA_2: $OD_{Sample} - OD_{Blank}$ (for GSSG).

2*: With GSSG as the standard, need to multiply by 2 when converting to GSH.

5**: Dilution multiple of sample in sample preparation step.

f: Dilution factor of sample before test.

ΔA_{412}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

m: the fresh weight of sample.

V_1: the volume of protein precipitator in sample preparation step of tissue sample.

1***: the cell number, 1×10^6.

V_2: the volume of protein precipitator in sample preparation step of cell sample.

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 (μmol/L)	3.50	12.80	22.50
%CV	1.0	0.4	0.4

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 (μmol/L)	3.50	12.80	22.50
%CV	4.1	3.6	4.0

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 97%.

	standard 1	standard 2	standard 3
Expected Conc. (μmol/L)	0.85	3.5	13.4
Observed Conc. (μmol/L)	0.8	3.4	12.6
recovery rate(%)	99	98	94

Sensitivity

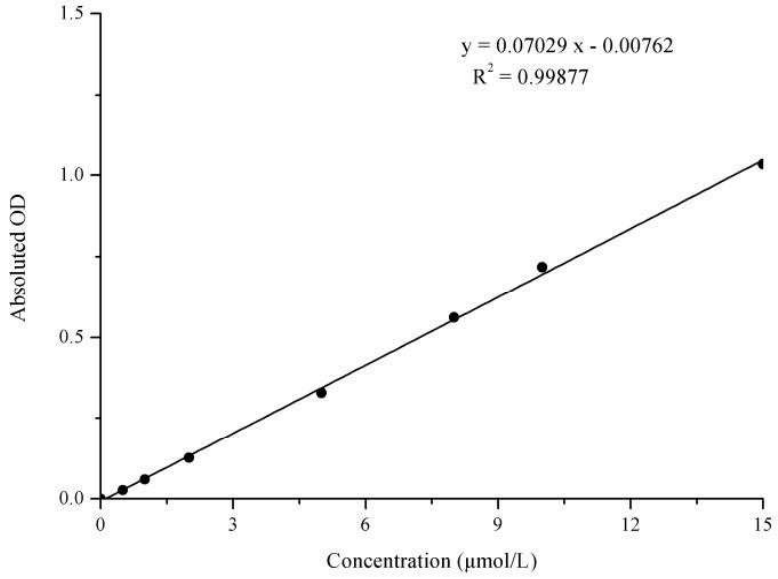
The analytical sensitivity of the assay is 0.36 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

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.

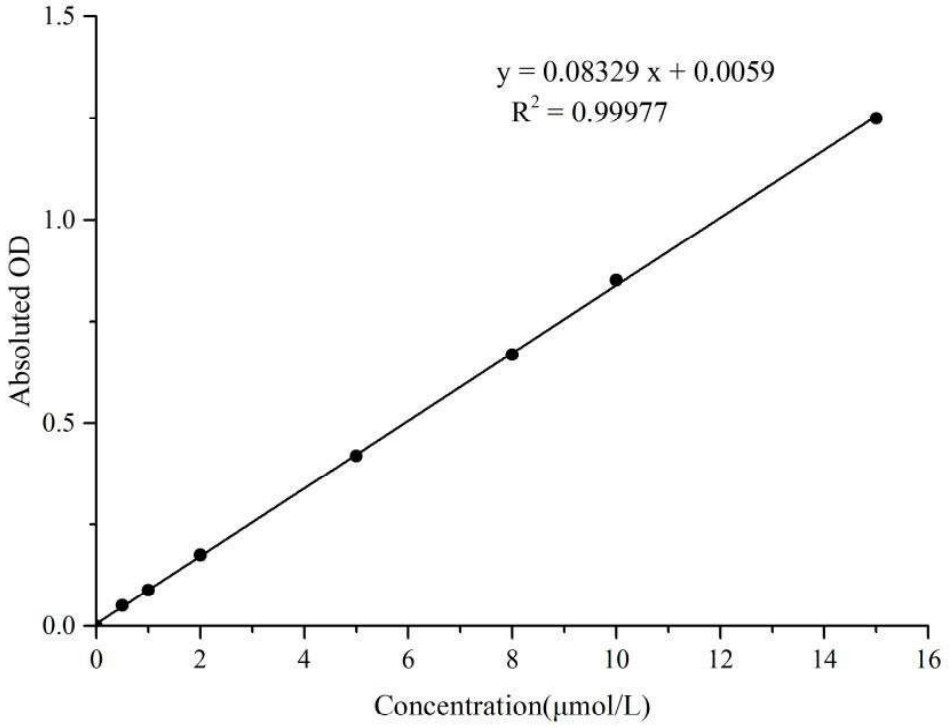
The standard curve of GSSG is as follows

Concentration (μmol/L)	0	0.5	1	2	5	8	10	15
Average OD	0.113	0.14	0.173	0.24	0.4415	0.6745	0.831	1.147
Absoluted OD	0	0.027	0.060	0.127	0.329	0.562	0.718	1.034



The standard curve of T-GSH is as follows

Concentration (μmol/L)	0	0.5	1	2	5	8	10	15
Average OD	0.105	0.156	0.194	0.281	0.524	0.773	0.957	1.355
Absoluted OD	0	0.051	0.089	0.176	0.419	0.668	0.852	1.250



Appendix Π Example Analysis

Example analysis :

Dilute 10% rat liver tissue homogenate with protein precipitator for 20 times, then take 10 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

The standard curve of T-GSH: y = 0.0858 x + 0.0064, the average OD value of the sample well is 1.159, the average OD value of the blank well is 0.114, the calculation result is:

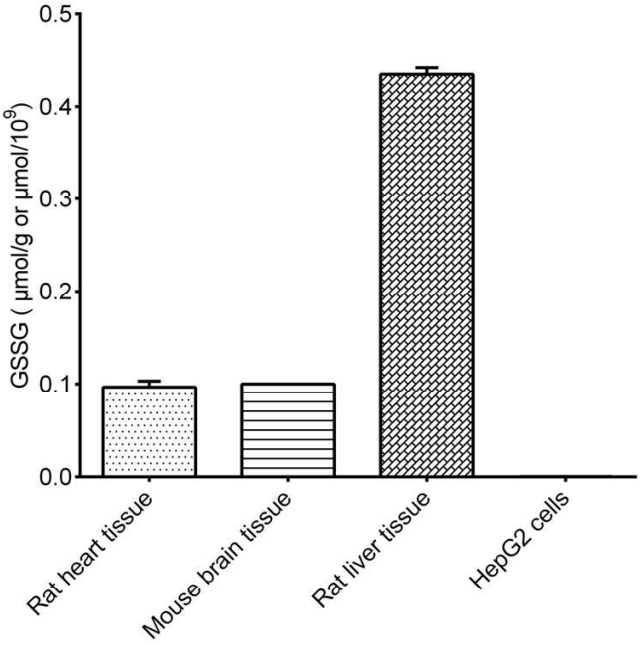
T-GSH (μmol/g) = (1.159 - 0.114 - 0.0064) ÷ 0.0858 × 2 ÷ 0.05 × 0.45 ×10^-3 × 20

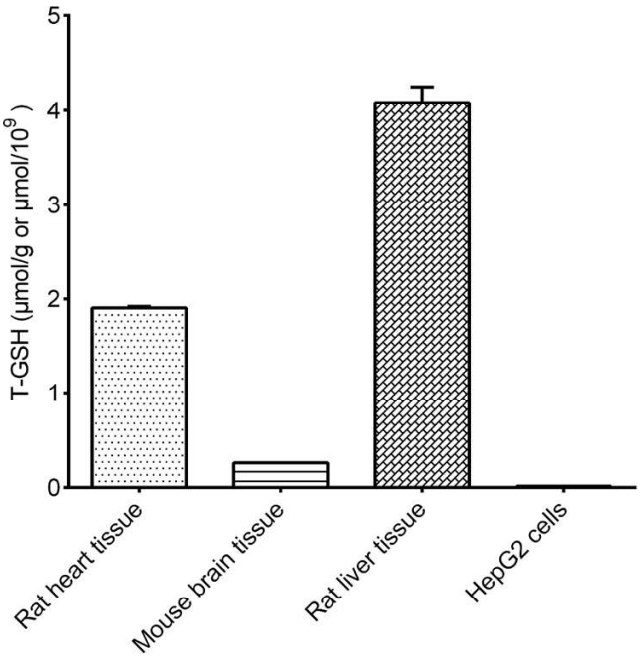
= 4.36 μmol/g

The standard curve of GSSG: y = 0.0717 x − 0.007, the average OD value of the sample well is 0.320, the average OD value of the blank well is 0.118, the calculation result is:

GSSG (μmol/g) = (0.320 - 0.118 + 0.007)÷ 0.0717 ÷ 0.05 × 0.45 × 10^{-3} × 20 = 0.52 μmol/g

Detect 10% rat heart tissue homogenate (dilute for 10 times), 10% mouse brain tissue homogenate (dilute for 2 times), 10% rat liver tissue homogenate (dilute for 20 times), HepG2 cells (dilute for 2 times) according to the protocol, the result is as follows :


0.5
0.4
0.3
0.2
0.1
0.0
GSSG (μmol/g or μmol/10^9)
Rat heart tissue
Mouse brain tissue
Rat liver tissue
HepG2 cells


5
4
3
2
1
0
T-GSH (μmol/g or μmol/10^9)
Rat heart tissue
Mouse brain tissue
Rat liver tissue
HepG2 cells

Appendix III Publications

1. Zhang L, Shi W Y,Jia Y , et al. Protective effects and mechanism of chemical- and plant-based selenocystine against cadmium-induced liver damage [J]. Journal of Hazardous Materials, 2024, 468 (Apr.15): 133812.1-133812.12. DOI: 10.1016/j.jhazmat.2024.133812.
2. Zhou Y, She R, Mei Z,et al. Crosstalk between ferroptosis and necroptosis in cerebral ischemia/reperfusion injury and Naotaifang formula exerts neuroprotective effect via HSP90-GCN2-ATF4 pathway[J].Phytomedicine, 2024, 130(000): 20. DOI: 10.1016/j.phymed.2024.155399.
3. Agnihotri P , Malik S , Saquib M ,et al.Exploring the impact of 2-hydroxyestradiol on heme oxygenase-1 to combat oxidative stress in rheumatoid arthritis[J]. International Journal of Biological Macromolecules, 2024, 283. DOI: 10.1016/j.ijbiomac.2024.137935.
4. Zhang Y , Feng R , Li H ,et al. CXCR4 influences PUFA desaturation and oxidative stress injury in experimental prostatitis mice by activating Fads2 via PPARγ [J]. Free Radical Biology & Medicine, 2024, 223 (000): 13. DOI: 10.1016/j.freeradbiomed.2024.07.015.
5. Y.-F. L , M.-Y. C , Chen Y ,et al.DHMBA, a molecule from Pacific oyster (Crassostrea gigas) alleviates AD pathology by inhibiting ubiquination of Nrf2[J].Food Bioscience, 2024:62.DOI:10.1016/j.fbio.2024.105259.

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.