(For scientific research use only, not for clinical diagnosis!)

Rat Glutathione Synthase (GSS) ELISA

Kit Instructions for Use Product No.: BY-

ER338976 Specifications: 48T/96T

Detection Range: 6.25 ng/mL– 200 ng/mL.

Sensitivity: The lowest detectable dose is less than 1.0 ng/mL.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Recovery rate: The recovery rate is between 85%-115%.

Specificity: This kit recognizes native and recombinant rat glutathione synthetase (GSS) and has no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.

Purpose: Used to detect the concentration of rat glutathione synthase (GSS) in samples such as serum, plasma, cell culture supernatant and tissue.

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 025-5229-8998 Sales department phone: 13914481711 Technical phone: 15950492658 Company website: www.byabscience.cn For the specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life.

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

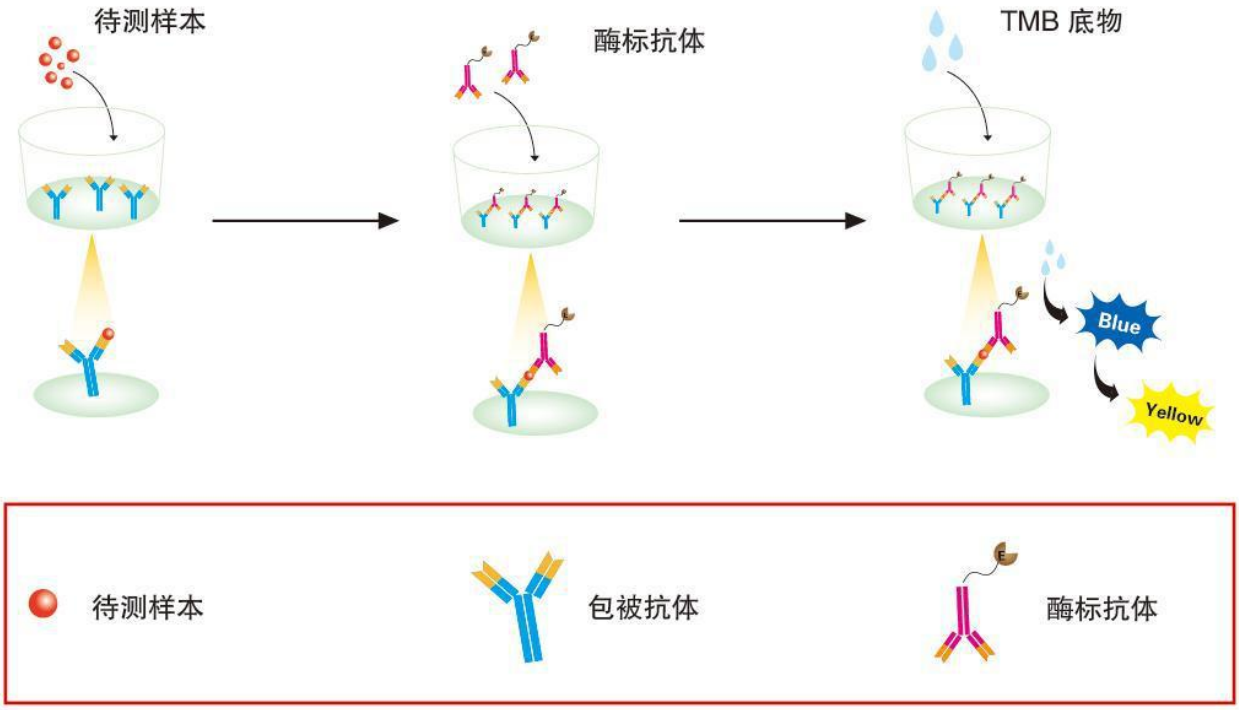
Nanjing BYabscience technology Co.,Ltd

Website: www.byabscience.cn **Official hotline: 025-5229-8998** **Supervision phone number:**

Experimental principle

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell microplate pre-coated with anti-rat glutathione synthase (GSS) antibody (solid-phase antibody), add rat glutathione synthase (GSS) calibrator and sample to be tested, and then Add HRP-labeled anti-rat glutathione synthase (GSS) antibody (enzyme-labeled antibody), and after incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen is formed on the solid surface of the microplate. -Sandwich complex of enzyme-labeled antibodies. Adding substrates A and B, the substrates are catalyzed by HRP to produce a blue product, which is finally converted to yellow under the action of the stop solution (acidic solution). The absorbance (OD value) was measured at a wavelength of 450 nm on a microplate reader. The absorbance (OD value) was positively correlated with the concentration of rat glutathione synthase (GSS) in the sample to be tested. The concentration of rat glutathione synthetase (GSS) in the sample can be calculated by fitting the calibrator curve.

Experimental schematic diagram



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Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.

Components	**48-well configuration**	**96-well configuration**	Store after opening
Pre-coated enzyme plate	48T	96T	**2-8℃14 days**
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	**2-8℃14 days**
sample diluent	3ml	6ml	2-8℃180 days
HRP labeled antibodies	5ml	10ml	**2-8℃14 days**
Chromogenic substrate A	3ml	6ml	2-8℃180 days
Chromogenic substrate B	3ml	6ml	2-8℃180 days
stop solution	3ml	6ml	2-8℃180 days
20×Lotion	15ml	25ml	2-8℃180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 200, 100, 50, 25, 12.5, 6.25 ng/mL.

Note: 1: Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

Prepare your own test equipment required for the test (not provided, but can assist in

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes, measuring cylinders, etc. for preparing

reagents 5) Distilled water or deionized

water Ionized water

6) Vortex shaker, microplate shaker

Notes 1) For scientific research use only, not for clinical diagnosis.

2) Use within the validity period marked on the kit. Expired products must not be used.

3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

6) The test results obtained by other methods are not directly comparable to the test results of this kit.

7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) 严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都必须在使用前达到室温 20-25℃。使用后立即冷藏保存试剂。

9) 洗板不正确可以导致不准确的结果。在加入底物前确保尽量吸干孔内液体。温育过程中不要让微孔干燥掉。

10) 消除板底残留的液体和手指印，否则影响 OD 值。

11）底物显色液应呈无色或很浅的颜色。

12）避免试剂和标本的交叉污染以免造成错误结果。

13）在储存和温育时避免强光直接照射。

14）检测使用的酶标仪需要安装能检测 450±10nm 波长的滤光片，光密度范围在 0-3.5 之间。建议使用时提前 15 分钟预热。

15）试验中所用的 EP 管和吸头均为一次性使用，严禁混用。

样品的准备和保存

以下只是列出样品采集和保存的一般指南。所有样本采集保存过程中，不得使用叠氮钠做为防腐剂。样品如果不立即分析，应分装后冷冻保存，且避免反复冻融。

细胞培养上清——离心去除沉淀，立即分析或分装后-20℃冷冻保存。

血清——用干净试管收集血液，室温凝固 30 分钟，离心 2000×g 20 分钟，收集血清。立即 分析或分装后-20℃冷冻保存。

血浆——采用肝素、柠檬酸盐或 EDTA 抗凝，抽血后 30 分钟内在 2-8℃离心 2000×g 20 分钟。为消除血小板的影响，建议在 2-8℃进一步离心 10000×g 10 分钟。立即分析或分装后-20℃冷冻保存。

细胞裂解液——对于贴壁细胞，去除培养液，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打数下，使裂解液和细胞充分接触。通常 10 秒后，细胞就会被裂解。对于悬浮细胞，离心收集细胞，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打把细胞吹散，用手指轻弹以充分裂解细胞。充分裂解后，10000—14000×g 离心 3-5 分钟， 取上清。立即分析或分装后-20℃冷冻保存。

Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or frozen and thawed repeatedly. Finally, centrifuge the homogenate at 5000 × g for 5 to 10 minutes, and take the supernatant for detection.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

Reagent preparation 1. Before use, all components must be rewarmed for at least 60 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water. 3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operating procedures: Return all reagents and components to room temperature first. For standards, quality control materials and samples, it is recommended to make duplicate holes.

1. Prepare the working solution of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Set up standard wells, 0 value wells, blank wells and sample wells. Add 50 μL of standards of different concentrations to each of the standard wells. Add 50 μL of sample diluent to the 0 value well. Do not add any to the blank well. Add 50 μL of the sample to be tested to the sample well. .

4. In addition to the blank wells, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to the standard wells, 0 value wells and sample wells.

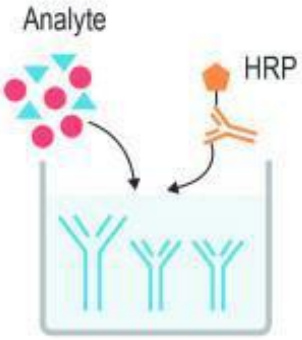
5. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

6. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, pat dry on absorbent paper, repeat this

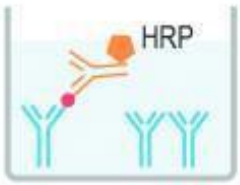
5 times. If you use an automatic plate washer, please wash the plate according to the plate washer operating procedure. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper. (Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 7. Mix substrates A and B at a volume of 1:1 Mix thoroughly and add 100 μL of substrate mixture to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

8. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450nm wavelength microplate reader.

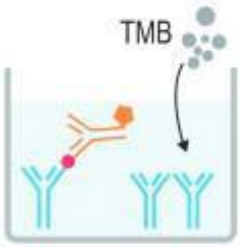
[Operation flow chart]



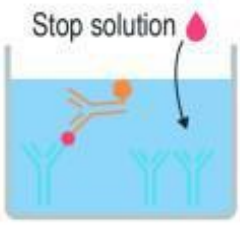
1. 对应板孔中加入50μL标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37°C孵育60分钟



2. 弃掉板内液体，洗板5次



3. 每孔加入底物A溶液50ul，底物B溶液50ul



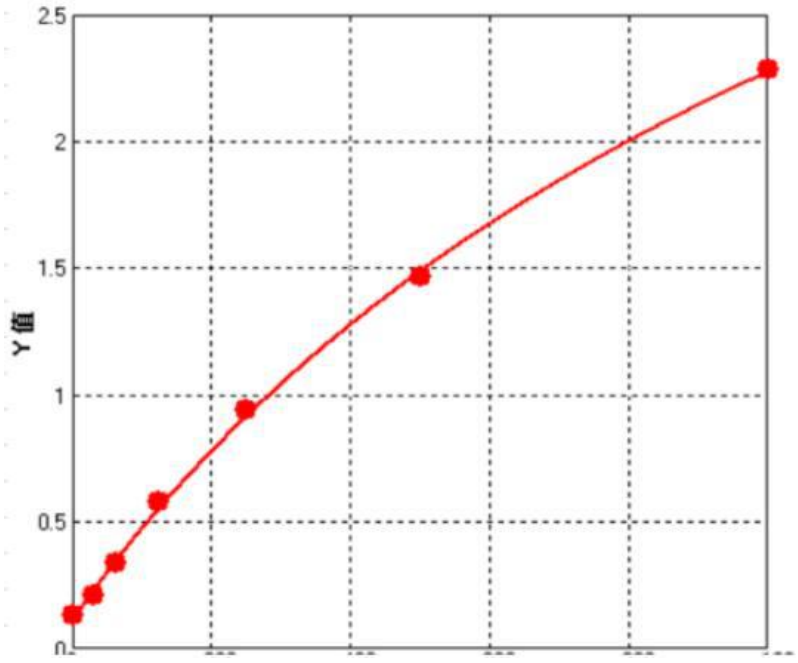
4. 每孔加入50μL终止液

5. 立即在450nm波长下读数，处理数据

Result calculation

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculate using ELISA Calc software. It is recommended to use four-parameter fitting for the standard curve, but it is not the only fitting method]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to determine the final value of the sample. concentration. Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only!



(Schematic diagram of the music, for reference only)

[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used laths and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information:

[Questions and Answers]

Problem description	Possible reasons	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washings and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

statement

1. Due to the current conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.

2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.

3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.

4. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.

5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may occur. Please read the instructions carefully and adjust the instrument before the experiment.

6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.

8. This kit has not been compared with similar kits from other manufacturers or products using different methods to detect the same target, so inconsistent test results cannot be ruled out.

9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.

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Website: www.byabscience.cn **Official hotline: 025-5229-8998** **Supervision phone number:**