



(仅供科研使用，不得用于临床诊断!)

兔颗粒酶 B(GZMB)ELISA 试剂盒

使用说明书 产品货号：BY-

ER773772 规格：48T/96T 检测范

围：31.25 pg/mL- 1000 pg/mL。

灵敏度：最低检出剂量小于 1.0 pg/mL。

精密度：批内变异系数 CV% 小于 10%；批间变异系数 CV% 小于 15%。

回收率：回收率在 85%-115% 之间。

特异性：本试剂盒识别天然和重组兔颗粒酶 B(GZMB)，与结构类似物无交叉。

稳定性：2℃-8℃ 保存，有效期 6 个月。

用途：用于检测血清、血浆、细胞培养上清液和组织等样本中兔颗粒酶 B(GZMB) 的浓度。

使用前请仔细阅读说明书。如果有任何问题，请通过以下方式联系我们：

官方热线：025-5229-8998 销售部电话：13914481711 技术电话：

15950492658 公司网址：www.byabscience.cn 具体保质期请见试剂盒外包

装标签。请在保质期内使用试剂盒。

联系时请提供产品货号、生产日期（见盒签），以便我们更高效为您服务。

Nanjing BYabscience technology Co.,Ltd

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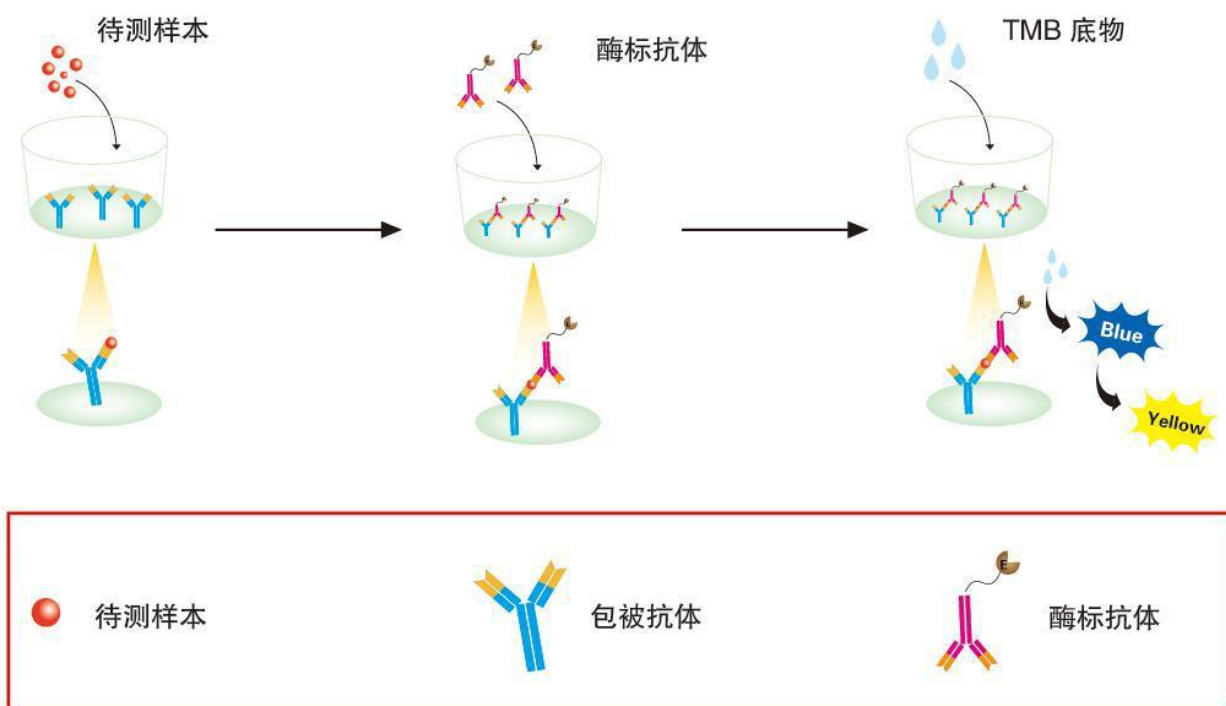
监督电话：15950492658



实验原理

本试剂盒采用双抗体夹心酶联免疫吸附试验（ELISA）。在预包被抗兔颗粒酶 B(GZMB) 抗体（固相抗体）的微孔酶标板中，加入兔颗粒酶 B(GZMB) 校准品和待测样本，再加入 HRP 标记的抗兔颗粒酶 B(GZMB) 抗体（酶标抗体），经过温育与充分洗涤，去除未结合的组分，在微孔板固相表面形成固相抗体-抗原-酶标抗体的夹心复合物。加底物 A 和 B，底物在 HRP 催化下，产生蓝色产物，在终止液（酸性溶液）作用下，最终转化为黄色。在酶标仪 450nm 波长上测定吸光度（OD 值），吸光度（OD 值）与待测样品中兔颗粒酶 B(GZMB) 的浓度正相关。拟合校准品曲线，可以计算出样本中兔颗粒酶 B(GZMB) 的浓度。

实验原理图





试剂盒组分与保存 未开封的试剂盒保存在 2-8 度，不得使用过期试剂盒。

组分	48 孔配置	96 孔配置	开封后储存
预包被酶标板	48T	96T	2-8℃14 天
标准品	0.3mL*6 管	0.3mL*6 管	2-8℃14 天
样本稀释液	3 ml	6 ml	2-8℃180 天
HRP 标记抗体	5 ml	10 ml	2-8℃14 天
显色底物 A	3 ml	6 ml	2-8℃180 天
显色底物 B	3 ml	6 ml	2-8℃180 天
终止液	3 ml	6 ml	2-8℃180 天
20×洗液	15 ml	25 ml	2-8℃180 天
封板膜	2 张	2 张	
说明书	1 份	1 份	
自封袋	1 个	1 个	

校准品浓度依次为：1000、500、250、125、62.5、31.25 pg/mL。

注意：1：使用前请检查试剂盒中试剂的标签和数量与表格是否一致。

2：如果试剂盒的组份需要再次使用，请确保上一次使用之后没有被污染。

3：酶标板单次未使用完，要谨记密封放到 2-8℃保存。

试验所需自备试验器材 (不提供，但可协助购买)

- 1) 能够检测 450 nm 吸光度的酶标仪
- 2) 移液器及枪头、加样槽
- 3) 37℃恒温箱或水浴锅
- 4) 准备试剂用的试管、离心管、量筒等
- 5) 蒸馏水或去离子水

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6) 涡旋振荡器、微孔板振荡器

注意事项 1) 仅供科研使用，不得用于临床诊断。

- 2) 在试剂盒标示的有效期内使用，过期产品不得使用。
- 3) 跟其他厂家的试剂盒或者组分不能混用，使用试剂盒配套的样品稀释液。
- 4) 如果样本值高于最高标准品浓度值，请将样本适当稀释后，再重新测定。
- 5) 待测样本中存在的人抗鼠等异嗜抗体会干扰检测结果，检测前，请排出该因素。
- 6) 通过其他方法得到的检测结果，与本试剂盒测定结果不具有直接的可比性。
- 7) 试验中请穿着实验服并戴乳胶手套做好防护工作。特别是检测血液或者其他体液样品时，请按国家生物试验室安全防护条例执行。
- 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) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.
- 10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
- 11) The substrate chromogenic solution should be colorless or very light in color.
- 12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.
- 13) Avoid direct exposure to strong light during storage and incubation.
- 14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of $450\pm 10\text{nm}$, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

15) The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

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Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide shall not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

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

according to the weight 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 the 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 repeatedly frozen and thawed. Finally, centrifuge the homogenate at $5000 \times g$ for 5 to 10 minutes, and take the supernatant for detection.

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

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**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. It is recommended to do duplicate holes for standards, quality control materials and samples.

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

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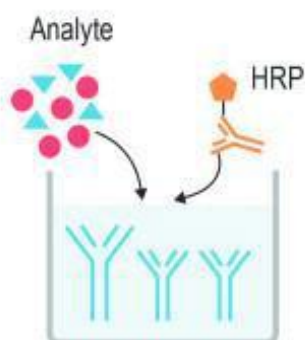
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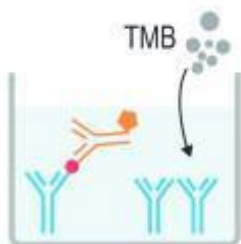
[Operation flow chart]



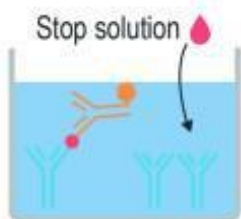
1. 对应板孔中加入50μL标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37°C孵育60分钟



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

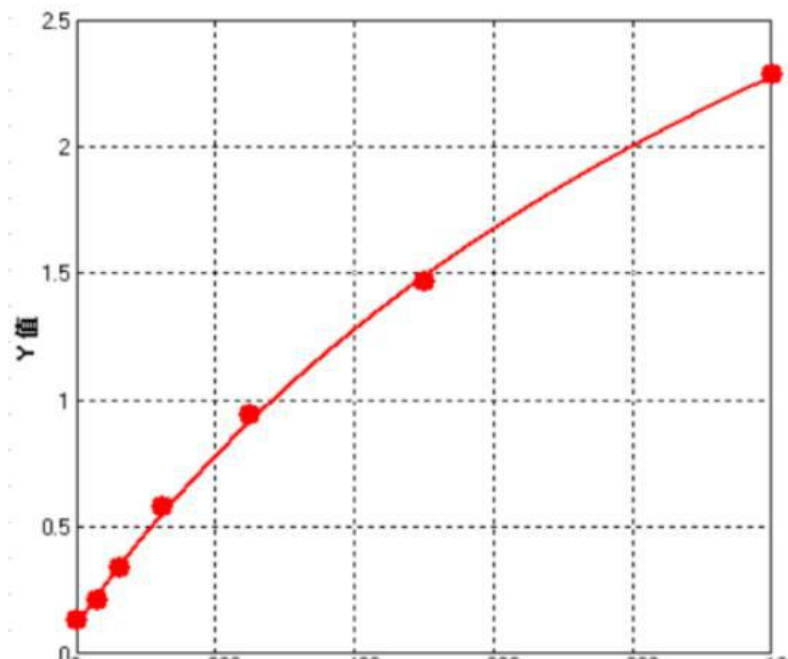


5. 立即在450nm波长下读数，处理数据



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)

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[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
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 washes 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

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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 result. 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 that detect the same target substance using different methods, 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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