(仅供科研使用，不得用于临床诊断!)

牛蓝舌病毒抗体(BLUV Ab)ELISA 试剂盒

使用说明书 产品货号：BY-EB777444 规格：48T/96T

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 联系邮箱：3224949330@qq.com 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。

试剂盒性能 物理性能：各液体组分澄清透明、无沉淀或者絮状物。微孔板铝箔袋应真空包装，无破损漏气。

阴性对照 OD 值：小于 0.2。

阳性对照 OD 值：大于 0.8。

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牛蓝舌病毒抗体(BLUV Ab)，与结构类似物无交叉。

稳定性：2℃-8℃保存，有效期 6 个月。

用途：用于定性检测血清、血浆、细胞培养上清液和组织等样本中是否含有牛蓝舌病毒抗体 (BLUV Ab)。

保质期：2℃-8℃保存，有效期 6 个月。

实验原理

试剂盒采用间接法酶联免疫吸附试验（ELISA）。往预先包被牛蓝舌病毒抗体(BLUV Ab)捕获抗原的包被微孔中，依次加入标本、阴性和阳性对照，再加入 HRP 标记的检测抗体，经过温育并彻底洗涤。用底物 TMB 显色，TMB 在过氧化物酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的牛蓝舌病毒抗体(BLUV Ab)呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），判定阴阳性。

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**
阴性对照	0.3mL	0.3mL	**2-8℃14 天**
阳性对照	0.3mL	0.3mL	**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	

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 6）涡旋振荡器、微孔板振荡器

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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±10nm, 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.

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 must 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 once 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 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 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 × 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.

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 and sample wells, and add 50 μL of standards of different concentrations to each standard well;

4. Add 50 μL of the sample to be tested into the sample well; do not add it to the blank well.

5. Except for the blank well, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to each well of the standard well and sample well, seal the reaction well with a sealing film, and keep the temperature at 37°C in a water bath or thermostatic oven. Incubate for 60 minutes.

6. Discard the liquid, pat dry on absorbent paper, fill each well with washing solution (350 μL), let it stand for 1 minute, shake off the washing solution, pat dry on absorbent paper, and repeat washing the plate 5 times (you can also use a plate washer to wash it) plate).

7. Add 50 μL each of substrates A and B to each well, and incubate at 37°C in the dark for 15 minutes.

8. Add 50 μL of stop solution to each well, and within 15 minutes, measure the OD value of each well at a wavelength of 450 nm.

[Interpretation of test results]

1. Negative control OD value: less than 0.2.

2. Positive control OD value: greater than 0.8.

3. Positive judgment (Cut-Off value): If the negative control OD value is +0.25, and the sample OD value is greater than the threshold, it is judged as positive, otherwise, it is negative.

[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 strips 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
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 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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