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

# Rabbit Vitamin B12 (VB12) ELISA

## Kit Instructions for Use

## Specifications: 48T/96T

**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 Contact email: 3224949330@qq.com Company website: www.byabscience.cn For specific shelf life, please see the reagents Box packaging label. 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:**

## Kit performance Detection range: 100 pg/mL-1600 pg/mL.

**Sensitivity: The lowest detectable dose is less than 10 pg/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 natural and recombinant rabbit vitamin B12 (VB12) and has no crossover with structural analogs.**

**Stability: Stored at 2℃-8℃, validity period is 6 months.**

**Purpose: Used to detect the concentration of rabbit vitamin B12 (VB12) in samples such as serum, plasma, cell culture supernatant and tissue. Shelf life: Stored at 2℃-8℃, valid for 6 months.**

## Experimental principle

The kit uses enzyme-linked immunoassay method. Biotin-labeled VB12 is used, and the purified anti-VB12 antibody is coated on the microwell plate. In the competitive inhibition reaction, a certain amount of solid-phase antibody is used to inhibit the competitive reaction with biotin-labeled VB12 and non-labeled antigen (calibrator or specimen). The antibody The amount of binding to biotin-labeled VB12 is inhibited by the amount of non-labeled antigen. The greater the amount of non-labeled antigen, the less the antibody will bind to biotin-labeled VB12, and vice versa. After the reaction is balanced, solid-phase antibody-biotinylation is formed. VB12, and then add enzyme-labeled avidin to form a solid-phase antibody-biotinylated VB12-enzyme-labeled-avidin complex. After adding substrate for color development, use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm. As the VB12 concentration increases, the OD value gradually decreases with a good linear

relationship. This kit has the characteristics of high sensitivity, strong specificity, good repeatability, simple and rapid operation, and has reliable detection performance for the reduction or increase of VB12 in serum.

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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 |
| biotinylated antigen | 3ml | 6ml | **2-8℃14 days** |
| HRP labeled avidin | 3ml | 6ml | 2-8℃180 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: 1600, 800, 400, 200, 100 and 0 pg/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

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6) Vortex oscillator and microplate oscillator.

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) 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) 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 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. 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. Take out the pre-coated plate from the sealed bag and set up a blank control well without adding any liquid; set up 2 wells for each calibrator and add 50 µl of the corresponding calibrator into each well; add the serum to be tested directly to each of the remaining detection holes. Or 50µl of quality control product.

4. Add 50 µl of biotinylated antigen to all wells except the blank well, mix well, attach sealing film, and incubate at 37°C for 60 minutes.

5. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

**(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.) 6. Add 50 µl of enzyme-labeled avidin to each well (blank (Excluding control wells), mix well, affix sealing film, and incubate at 37°C for 30 minutes.**

7. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

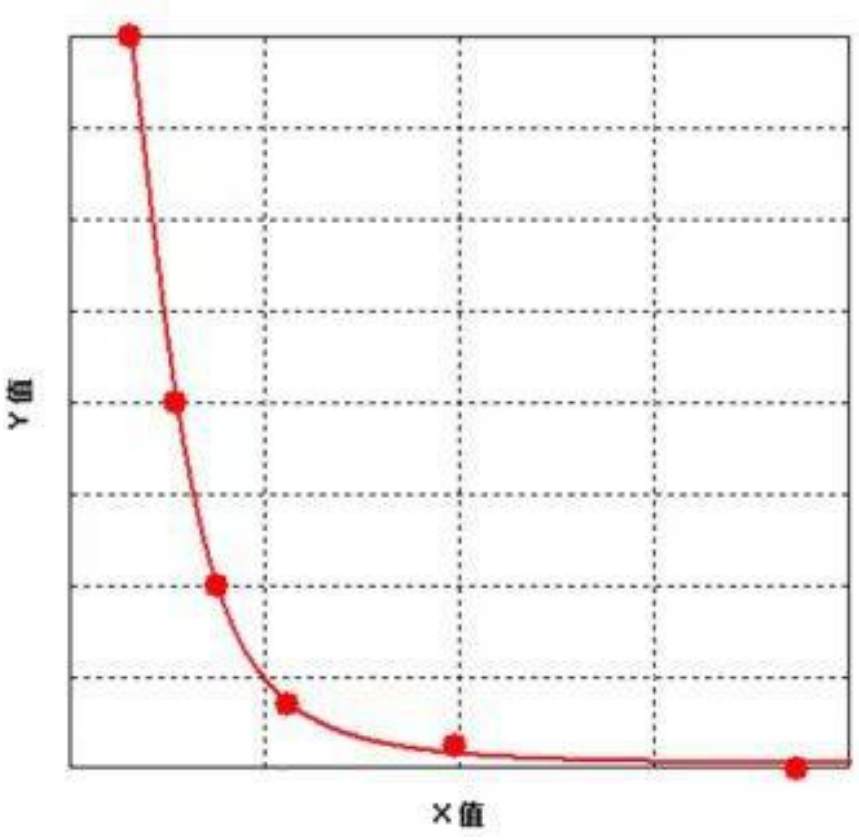
8. Add 50 µl of chromogen A and 50 µl of chromogen B to each well. After shaking and mixing, place at 37℃ to develop color in the dark for 15 minutes. Add 50 µl of stop solution to each well.

9. Use a microplate reader to read, take the wavelength of 450nm, first use the blank control well to adjust the zero point, and then measure the optical density value (OD value) of each well.

## 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. [Calculation using ELISA Calc software]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample. 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 washes and the amount of liquid added to each hole |
| Very weak or colorless | Incubation time too short | Ensure adequate incubation time |
| | Experimental temperature is incorrect | Use recommended experimental temperatures |
| | Insufficient reagent volume or missing addition | Check the liquid aspiration and addition process to ensure that all reagents are added in sufficient |
| | 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 |